A Thesis Submitted for the Degree of Doctor of Philosophy at

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Utilising the patchy distribution of slugs to optimise targeting of control; improved sustainability through precision application

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Declaration

I declare that this thesis has been composed solely by myself and that it has not been submitted, in whole or in part, in any previous application for a degree. Except where states otherwise by reference or acknowledgment, the work presented is entirely my own.

Emily Forbes

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Abstract

The grey field slug (*Deroceras reticulatum*) is an agricultural pest, causing economic damage to a range of crops in the UK. Legislation has led to a reduction of registered active ingredients and increased pressure to reduce pesticide usage. Discontinuous distributions of slugs in arable fields offers the potential to target control applications on patches of slugs, reducing pesticide use whilst maintaining efficiency. This thesis investigates the stability of patches and methods for locating them. Significant aggregations of slugs were found at all field sites with stable areas of higher slug densities occurring in the same area of the field at all five fields sites during the 2015-16 season. In the subsequent two seasons slug numbers were lower, however, similar patterns of stability were observed in the fields with the largest populations. Stability of patches between seasons requires further work. Alternative methods of locating areas of higher slug densities were investigated. Using crop damage from grazing was not found to be suitable, the highest correlation between slug numbers and damage was r = 0.52, no positive correlation was found in the field with the largest population. Using soil characteristics was also investigated with organic matter, pH, bulk density and soil texture found to be significantly different at some field sites within and outside of slug patches, providing potential candidates for further investigation. A method of identifying individual slugs was developed to improve understanding of patch formation. Radio frequency identification tags were used to track slugs in the field over two five-week periods. Slugs were found to remain close to their release point. The maximum distance moved from the point of release was 78.7 cm in April 2017 and 101.9 cm in November 2017. The combination of results from this work suggests there is strong potential for targeting molluscicides to areas of higher slug densities.

Contents

List of Tables

List of Figures

Abbreviations

°C	Degree Celsius
%	Percentage
ANOVA	Analysis of Variance
cm	Centimetre(s)
CPD	Continuing Professional Development
DAT	Defined Area Trap
EU	European Union
g	Gram(s)
GPS	Global Positioning System
h	Hour(s)
IBM	Individual Based Modelling
IPM	Integrated Pest Management
IPMP	Integrated Pest Management Plans
m	Metre(s)
ml	Millilitre(s)
mm	Millimetre(s)
µg	Microgram(s)
µm	Micro metre(s)
NRoSO	National Register of Sprayer Operators
OSR	Oilseed Rape
PAD	Pesticide Authorisation Directive
PPP	Plant Protection Product
RFID	Radio Frequency Identification
spp	species
UK	United Kingdom
UV	Ultra Violet
WFD	Water Framework Directive

1. Chapter 1. Literature review

1.1. General biology of slugs

1.1.1. Taxonomy

All terrestrial slug species evolved from terrestrial snails through several separate evolutionary events (Rowson *et al.*, 2014). In the UK there are 43 species of terrestrial slug (Rowson *et al.,* 2014), which belong to seven families (Agriolimacidae, Arionidae, Boettgerillidae, Milacidae, Limacidae, Testacellidae and Trigonochlamydidae) in the order Stylommatophora, class Gastropoda and phylum Mollusca (Rowson *et al*., 2014). Within the order Stylommatophora there are many pest species belonging to different families. Slugs are a worldwide crop pest, with examples including *Sarasinula plebeian* (family Veronicellidae) in dry beans in Central America, *Urocyclus flavescens* (family Urocyclidae) in bananas in South Africa and *Deroceras reticulatum* (Müller), *Arion hortensis agg*. and *Tandonia budapestensis* in cereal crops in Western Europe (Barker, 2002). Pest species in the UK are found in four of the six native families, Agriolimacidae, Arionidae, Limacidae and Milacidae, *D. reticulatum* is in the Agriolimacidae family (Rowson *et al.,* 2004). *Deroceras reticulatum* is the most economically important slug pest in Europe and also damages a wide range of agricultural crops in Asia and USA (Kozlowski and Jaskulska 2014; Ramsden *et al.*, 2017).

1.1.2. Slug morphology and physiology

Families of slugs are characterised by several morphological differences. For example, the extent to which the shell has reduced varies; in the Testacellidae a reduced external shell is still visible on the dorsal body wall, while in the Agriolimacidae, Limacidae and Millacidae the shell has become a small plate under the mantle, whereas in the Arionidae only calcareous granules remain and in the Boettgerillidae the reduction is even more extreme (South, 1992; Rowson *et al.,* 2014). Another difference between families is the presence of a keel, in the Arionidae there is no keel present, in contrast the Millacidae and Boettgerillidae have a keel running from the rear of the tail to just behind the mantle and the Limacidae and Agriolimacidae have a short keel from the rear of the tail finishing half way to the rear of the mantle (Rowson *et al*., 2014).

The mantle (Figure 1.1(A)), protects the slug's organs, stomach, reproductive organs and anus ((Figure 1.1(B)), which are asymmetrical, to the right, in common with their snail ancestors (Rowson *et al.,* 2014). There is also a small opening, the breathing pore, on the right-hand side of the mantle called the pneumostome (Figure 1.1(A)). Slugs have two sets of tentacles, the longer posterior optical tentacles and the shorter anterior sensory tentacles (Figure 1.1(A)) (South, 1992). The reduction of the shell has allowed slugs to exploit different niches to their snail ancestors, the production of a hard calcareous shell requires large amounts of specific resources restricting the soils and diets snails can survive on, and without a large shell slugs are able to move faster than snails and access smaller spaces (Rowson *et al.,* 2014). The disadvantage of the reduction of the shell is that slugs have less protection from predators and lose more water through evaporation from their skin, as they are unable to regulate their own body moisture they therefore rely on moisture in their environment (South, 1992).

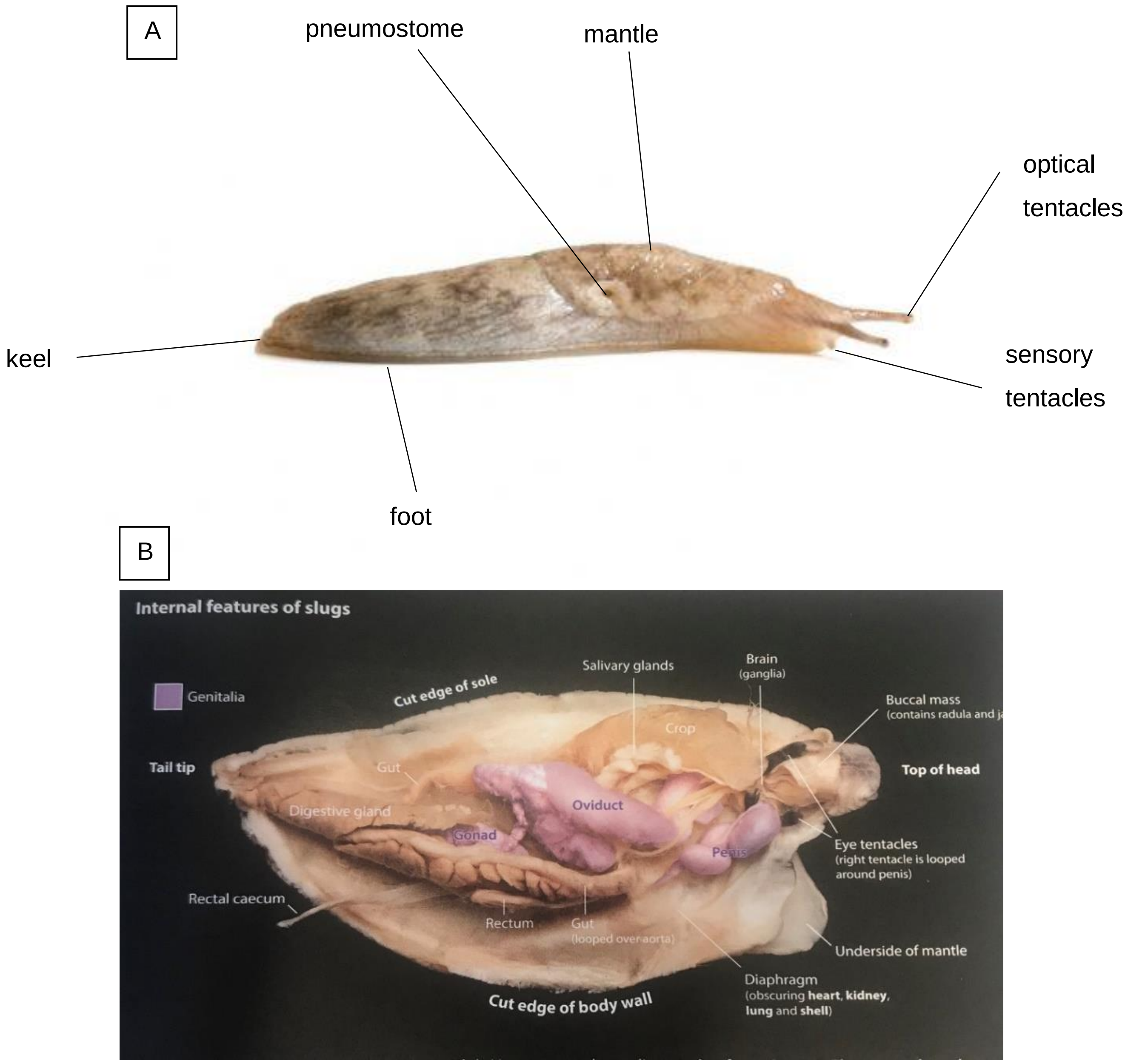


Figure 1.1. The morphology of *Deroceras reticulatum*. (A) external morphology (John Innes Centre, 2019); (B) internal anatomy (Rowson *et al*., 2014).

1.1.3. UK agricultural pest species

Of the species recorded in the UK, *D. reticulatum*, *Arion spp.* and *T. budapestensis* account for the majority of damage resulting from slug activity in arable crops (Rowson *et al.,* 2014). Distributed throughout the UK, *D. reticulatum* is an opportunistic species that reproduces rapidly to exploit the disturbance of soil in arable fields. It is the most economically damaging species of slug affecting cereal and oilseed rape (OSR) crops (Kemp and Newell, 1987; South, 1992), with *T. budapestensis* being the main threat to potatoes (Thomas, 1947, Stephenson 1967, South, 1992). Much of the research related to slugs and crop damage was carried out during the 1980s and 1990s, however, more recent publications (Kozlowski and Jaskulska, 2014; Rowson *et al.,* 2014; Ramsden *et al.,* 2017), have also contributed to the development of advice for farmers (AHDB, 2016) and these species continue to be considered the biggest threat to these crops in the UK.

1.1.4. Deroceras reticulatum (Müller)

The grey field slug, *D. reticulatum* (previously named *Agriolimax reticulatus* (Müller) is the most economically important slug species, accounting for the majority of damage to crops in the UK (Ramsden *et al.*, 2017). *Deroceras reticulatum* is indigenous to Europe but has been introduced to other parts of the world including USA, South America, central Asia and New Zealand (Yildirim and Kebapçi, 2004). *Deroceras reticulatum* varies in size according to habitat, with the largest individuals being approximately 5 cm in length when fully extended (Rowson *et al.,* 2014). Although it is difficult to distinguish *D. reticulatum* from other *Deroceras* species (particularly *D. agreste*) based solely on external characteristics, the geographical distribution is very different, *D. reticulatum* is found in commonly disturbed habitats whereas *D. agreste* is mainly found in woodland. *Deroceras reticulatum* is omnivorous but shows a preference for live or decaying plant material (South, 1992). The life span of *D. reticulatum* in their natural habitat is approximately 1 year (South, 1989). Hereafter the term “slugs” will refer to *D. reticulatum* unless specified otherwise.

1.1.5. Factors affecting slug activity

Slugs are more active at night (Wareing and Bailey, 1985; Hommay *et al.,* 1998; South, 1992). During periods of inactivity slugs will seek out refuges under stones, at the base of plants or by moving down the soil profile using cracks. Hommay *et al.* (1998) carried out two laboratory experiments to investigate locomotor activity in relation to refuge traps. The first used a photoperiod of 12 hours: 12 hours (light: dark) with the temperature maintained between 15 and 18°C while a second experiment had a photoperiod of 10 hours: 14 hours (light: dark) at a constant temperature of 14°C. In both experiments slugs left the refuges and activity increased rapidly soon after it became dark and decreased shortly after it became light, when slugs returned to a refuge. A feeding peak was observed 1-2 hours after it had become dark and a mating peak occurred during the middle part of the scotophase (Hommay *et al.,* 1998). Research investigating patterns of activity in constant temperatures and light conditions has demonstrated that even in an absence of environmental cues such as changing light levels or temperature, patterns of daily activity can persist (South, 1992). These experiments suggest that photoperiod rather than temperature is the primary driver of the onset of slug activity, although under field conditions diurnal temperature cycles mirror cyclical changes to light levels and interactions may occur.

Previous work on the effect of temperature and daylength under controlled moisture levels found that daylength had a significant effect on the optimum temperature for locomotor activity but not feeding of *D. reticulatum* (Wareing and Bailey, 1985). Under short daylengths (10 hours light: 14 hours dark) the optimum temperature for locomotor activity was 13°C increasing to 17°C under longer daylengths (14 hours light: 10 hours dark), whilst the optimum temperature for feeding remained at 14°C in both conditions. The study also showed both rate and direction of change of physical conditions were also important. With a daytime temperature of 12°C, cooling temperatures in the dark period stimulated locomotion and feeding activity, whereas rising temperatures supressed activity.

In combination, the results of the work carried out by Hommay *et al.* (1998) and Wareing and Bailey (1985) suggest that day light is the main factor in determining the onset of activity but temperature also has a role to play in the level of activity. *Deroceras reticulatum* is able to remain active and cause crop damage in a wider range of temperatures than most other slug species. For example, *D. reticulatum* have been recorded feeding at temperatures as low as 0.8°C, while other slug species were found to be inactive below 5°C (Mellanby, 1961).

Although daylength and temperature have been shown to influence the timing of slug activity, moisture is also widely acknowledged as affecting the number of slugs active on the soil surface. Daylength is a variable with known daily and seasonal cycles, while temperature and moisture fluctuate over shorter time periods. Whilst seasons may have typical weather conditions within seasons there is daily variation in temperature and soil moisture which can influence slug activity.

Models of slug populations have used parameters such as soil moisture, air temperature and leaf area index (Shirley *et al.,* 2001) and rainfall and temperature (Choi *et al.,* 2004). Whilst research has confirmed temperature and moisture play an important role in daily slug activity and long term population numbers, the precise combination of factors associated with slug abundance are often disputed. This could be in part due to factors which vary between study sites and were not considered in all experiments, such as cultivations, soil texture and predator abundance. Predation by carabids was shown to influence slug populations (Schley and Bees, 2003) and a model which included parameters relating to egg production, development of juveniles and mortality predicted population dynamics over a 3.5-year period more accurately than those based solely on weather data (Choi *et al.,* 2006).

1.1.6. Slug reproduction

Deroceras reticulatum has two main peaks of reproductive activity, one in spring and one in autumn, however, it is an opportunistic species which reproduces whenever favourable mild, wet conditions occur, resulting in overlapping generations/life stages throughout the year (Port and Port, 1986). In contrast, *Arion spp.* and *T. budapestensis* have an annual life cycle with a single peak of reproductive activity occurring in late spring to early summer (South, 1992). These alternative strategies allow the different species to exploit their niches, with *D. reticulatum* being found mainly in disturbed environments, and *Arion* spp. primarily inhabiting more stable environments (South, 1989). The annual pattern of *D. reticulatum* with two reproductive peaks, coupled with their response to environmental cues such as temperature (rather than photoperiod), enables populations to increase rapidly when environmental conditions are favourable, a response that is critical in disturbed environments.

Although slug eggs are widely known to be susceptible to desiccation, there is evidence that *D. reticulatum* can mitigate for this by adjusting the number of eggs laid depending on the environmental temperature and moisture conditions (Willis *et al*., 2008). This again allows reproductive efforts to exploit the disturbed environment to maximise population increases when conditions are optimum. Egg production in other species, such as *Limax maximus* and *Ariolimax columbianus* which inhabit more stable environments, is related to the photoperiod (Rollo, 1983; Kozłowski and Sionek, 2000). Laboratory experiments carried out by Hommay *et al.* (2001) also showed a response to daylength, with slugs producing more eggs in longer photoperiods. The data may however reflect an effect of temperature changes rather than photoperiod, as in the shorter photoperiods the temperature was increased in the dark whereas in the longer photoperiods the temperature was decreased during the dark.

Whilst eggs are susceptible to desiccation, the juveniles have the lowest rates of overwintering survival; eggs which fail to hatch by the end of the autumn may remain dormant until the following spring (Shley and Bees, 2002). Most slug species have three post-hatching life stages; infantile (rapid growth), juvenile (medium growth) and adult (minimal growth) (South, 1982; Hommay *et al.,* 2001). *Deroceras reticulatum* and *Arion circumscriptus* (another species which inhabits disturbed ground (Rowson *et al.,* 2014)) only have two post-hatching life stages, namely the juvenile (rapid growth) and adult (slow growth) stages (South, 1982).

Slugs are hermaphrodites. During the juvenile stage *D. reticulatum* are typically males (Schley and Bees, 2002), with older adults taking the female role. In the absence of juveniles, adult slugs can also take the male role to prevent a reduction in reproduction and so population size (Schley and Bees, 2002). Juveniles are not only vulnerable during over-wintering due to adverse environmental conditions, but are also at risk from predation by carabids (Schely and Bees, 2003). The faster growth rate during the juvenile stage of *D. reticulatum* reduces the time that young slugs are at risk due to extreme environmental conditions or predation.

1.1.7. Slug population dynamics

The effect of weather-related environmental factors on slug activity and the long-term effect on their populations have previously been discussed. However, the variation in the number of slugs between fields is controlled by more stable factors, for instance, soil organic matter, pH and soil type affect slug population densities (South, 1992). In addition to providing a food source for slugs (Carrick, 1942), decomposing plant material influences other soil properties such as water holding capacity, soil structure and pH. Increased organic matter content can increase the water holding capacity of soil, meaning longer moisture retention during dry periods (Franzluebbers, 2002). Slugs are dependent on their environment for moisture as they are unable to regulate their own body moisture (South, 1992). Organic matter also improves the soil structure and stability of sandy soils and increases soil aggregation, decreasing the bulk density (Keller and Håkansson, 2010), creating refuges for soil dwelling organisms (Franzluebbers, 2002).

There are mixed results in the literature regarding the effect of pH on slug abundance. The early research investigating the relationship between molluscs and pH used snails as the study species and showed a higher number of species present in soils with pH 6 or above (Atkins and Lebour, 1923). It is likely that slugs have a similar preference for neutral to alkaline soils. Slugs have several requirements for calcium carbonate, in the reduced calcareous shell under the mantle (Figure 1.1), an outer layer of calcium carbonate surrounding their eggs and granules of calcium carbonate within their slime (South, 1992). Calcium carbonate is found in more alkaline soils. A study in Iberia found higher *D. reticulatum* populations in areas with soils with high pH and calcium levels (Ondina *et al.,* 2004).

Soils with a high clay content tend to form clods and cracks when they dry out (Hillel, 2008). The clods and cracks in the soil provide refuges for slugs, which rely on them as an environment to assist in maintaining body moisture levels and for shelter (South, 1992). Soil texture is widely considered to be an important factor in determining the slug population size in arable fields. For example, damage records from the 1957 and 1958 growing seasons in East Anglia showed a high correlation between crop losses in winter wheat and the soil type, the majority of damage occurring on clay loam soil, with no reports of damage on sandy soil (Gould, 1961). Ondina *et al.* (2004) made similar observations during an investigation on slug population distributions in Iberia, where *D. reticulatum* showed a preference for soils with high silt and clay proportions. In recent literature produced by AHDB (2018) soil type is identified as a risk factor for slugs, with soils that have high clay and silt content being considered more prone to greater slug numbers.

1.1.8. Discontinuous distribution

Discontinuous distribution of slugs in arable fields is widely reported in the literature (South, 1992; Bohan *et al.,* 2000a; Archard *et al.,* 2004; Mueller-Warrant *et al.*, 2014). For example, Bohan *et al.* (2000a) demonstrated aggregation of juvenile *D. reticulatum* and *Arion intermedius* and Archard *et al.* (2004) identified areas (patches) with higher slug numbers using both soil samples (25 x 25 x 10 cm) and surface traps, positioned 16 m apart in a 6 by 4 rectangular grid. Subsequent work has indicated that although similar sampling grids consistently identify such slug patches, reducing the inter-trap distance to 10 m or less increases the accuracy by which patch location can be established (Petrovskaya e*t al*., 2018). Fewer studies have investigated environmental or behavioural mechanisms determining how and where such patches form or their temporal and spatial stability and this remains a significant constraint on our ability to use such patches for more accurate targeting of molluscicide application to reduce the volume of pesticides applied.

1.1.9. Mucus trail following

Trail following occurs in many different species from mammals to insects and gastropods (Ng *et al.,* 2013) and can serve several different functions including homing, mate location, energy saving or aggregation. Mucus production is costly, and utilising trails which have recently been laid by another individual can reduce the amount of mucus produced by the following slug by as much as 73 % making locomotion more efficient (Davies and Blackwell, 2007).

In a review of trail following in gastropods Ng *et al.* (2013) concluded that trail following is not the primary mechanism in homing behaviour but can aid it. Rollo and Wellington (1981) investigated mucus trail following in relation to homing behaviour in nine different species of slugs and snails, including *D. reticulatum*. Only a small percentage of the mucus trail following that they reported was found to be for homing, with olfactory cues from refuges (mainly from faeces) being preferentially used. In field experiments observing eight slug species, *D. reticulatum* were observed travelling directly to a shelter up to 1.5 m away without following slime trails. Whilst travelling to a refuge, slugs were recorded lifting the anterior part of their body and swaying their head with their optical tentacles spread widely and then narrowing as they approached the refuge. This behaviour suggested that olfactory cues inside the shelter were influencing the direction the slugs travelled rather than slime trails (Rollo and Wellington, 1981). In bad weather (wind and rain), when such cues would be weaker, similar behaviour was observed but rather than heading directly towards the refuge they circled in increasingly smaller circles. Other instances of trail following resulted in mating or attacking other slugs (Rollo and Wellington, 1981).

In many gastropod species trail following has been shown to be species specific and often results in mating. In species which are not hermaphrodite, the different sexes can also be distinguished (Ng *et al.,* 2013). For example, in the marine snails *Littoraria ardouiniana* and *L. melanostoma,* it has been shown that males are able to both identify females from cues in the mucus trail and the direction of the trail, thus increasing mating success (Ng *et al.*, 2013). The mechanism for directional trail following is unknown but there are several possibilities; a directional cue in the structure of the mucus, chemical cues in the mucus which deteriorate over time creating a gradient, or an olfactory cue from the slug which laid the trail (Cook, 2001). There is evidence that trail following for mating may be part of pre-courtship behaviour with both the trail layer and trail follower both being active participants (Reise, 2007). There may also be a benefit of trail following in that it will lead to aggregations which provide benefits in the form of protection from predators and reduced desiccation through huddling (Ng *et al.,* 2013).

Although trail following may vary between species, directional trail following responses have been recorded in *D. reticulatum*, with one study demonstrating that in more than 90 % of observed encounters, individuals that met a slime trail were able to identify the direction in which the trail was laid and turned to follow the slug that laid it (Wareing, 1986). The slug following the trail moved faster than the slug laying the trail and caught the trail layer in more than 50 % of cases. In all cases where the slug caught up with the slug which had originally laid the trail courtship behaviour resulted. The age of the trail encountered significantly affected the response elicited. Those which had been laid up to 6.5 hours prior to the encounter were followed, whereas there was no response to older trails (up to 3 days old). It is not known whether these trails were not detected or ignored as following older trails would be unlikely to result in finding a mate (Wareing, 1986). Understanding the biological significance of trail following and the tendency for an individual to follow a trail of another individual of the same species is important in relation to patch formation. Increased trail following will lead to increased aggregation (and thus coherence of patches), reinforcing any responses resulting in preference for specific environmental characteristics, as well as compounding aggregations in areas of preferred environmental conditions (Cook, 2001).

1.1.10. Homing behaviour

Both homing and aggregating behaviours may contribute to patch stability. Laboratory studies have demonstrated homing behaviour in slugs (Hommay *et al.,* 1998; Cook, 1979; Gelperin, 1974) although homing behaviour observed in the laboratory may not be representative of behaviour in the field. In laboratory experiments, the available space is small, with a limited choice of refugia, and conditions are uniform when compared to the field. Rollo and Wellington (1981) assessed the homing behaviour of several species of slugs, including *D. reticulatum* under field and laboratory conditions. In field studies individual slugs have been observed returning to the refuge used the previous night via a different route to the one they left by. When refuges have been artificially manipulated (i.e. relocated), individual slugs have been observed returning to the same refuge as they previously used in its new position (Rollo and Wellington, 1981). Other studies have shown when mucous trails have been removed, some slug species are still able to locate the same refuge, although these responses have been found to vary between species (Ng *et al.,* 2013). There is evidence that some species will use air-borne chemical cues, potentially deposited in faeces, to find their way back to a refuge but in some circumstances e.g. a change in wind direction, they will switch to using contact chemoreception to follow mucus trails (Ng *et al.,* 2013). Rollo and Wellington (1981) manipulated refuges, with carrot and faeces enclosed in some and only carrot in others. Significantly more *Arion ater* were found in the refuges containing faeces than carrot alone. These results suggest that the slugs are following olfactory cues, potentially in faeces in order to return to the shelter. Further evidence that slugs use olfactory cues to return to their shelter is provided by Cook (1980) who observed significantly more slugs (*Limax pseudoflavus*) returning upwind to refuges than would be expected if the movement was random, whereas there was no significant difference from random movement when leaving the shelter. The behaviour observed by Cook (1980) was not homing because it was not the same individuals returning to specific refuges each night. Individual slugs were more likely to use a refuge that others of the same species had utilised the previous night, suggesting that individuals were not following their own individual signal but rather a species-specific signal. The tendency to return to the same refuge, or even a refuge in the same area used by individuals of the same species, will contribute to the stability of patches.

Rollo and Wellington (1981) reported seasonal variations in the tendency for slugs to return to their previous refuge. There was also a tendency for slugs to use the same refuge in dry conditions, especially during summer months when the hours of darkness were limited and less time was available to seek refuges whereas in the autumn there was more movement of slugs between refuges (Rollo and Wellington, 1981). All slugs showed the ability to seek out alternative refuges when conditions became less favourable, for example when food became scare or a shelter became flooded, too dry or there were dead slugs present (Rollo and Wellington, 1981). A review of homing behaviour in gastropods (Cook, 2001) found that homing behaviours were more frequently observed in laboratory experiments compared to field studies. One possible explanation for this is that in field conditions suitable refuges are more abundant. The use of the same refuges during the summer or dry periods would increase the stability of patch location observed during these periods and the increase of movement between refuges in the autumn may make patch location less stable at a critical time for molluscicide applications.

1.2. Slugs as a crop pest

Slugs cause damage to a range of crops including cereals, oilseed rape, potatoes, asparagus, brussel sprouts, carrots and lettuce. The damage caused by slugs can either affect the yield or make the crop unmarketable through cosmetic damage (AHDB, 2016). This thesis will concentrate on cereals and oilseed rape crops, the damage caused by slugs to these crops is discussed in more detail below.

1.2.1. Cereals

Damage caused by slugs varies between crops. Wheat and other cereals are most susceptible to damage before germination when seed hollowing caused by slugs can result in poor germination leading to poor crop establishment (South, 1992). Damage can also occur after germination from grazing on young shoots up to GS21 (AHDB, 2016). Wheat can recover from seed damage, provided it does not prevent germination. Feeding by slugs on mature plants is not considered to be of significant economic importance (Port and Port, 1986). Although few comparisons of variation in susceptibility of modern wheat cultivars have been conducted there is no evidence of significant differences occurring (ADAS, 2010; Cook *et al*., 1996).

1.2.2. Oilseed rape

Oilseed rape is most vulnerable during the establishment phase (between sowing and the four true leaf stage), with most damage caused by leaf shredding rather than by seed hollowing (AHDB, 2016). There is evidence that some OSR varieties are more susceptible, with those having higher concentrations of glucosinolates being less vulnerable to damage from slugs. Experiments investigating nine commercial cultivars of OSR found a strong inverse relationship between slug damage and the levels of glucosinolates (Glen *et al.,* 1990a). Varietal differences are not commonly observed as OSR breeding targets lower glucosinolate level varieties due to the toxicity to livestock (Bellostas *et al.*, 2007).

1.2.3. Slug numbers and damage

Correlations between slug population densities and resultant crop damage were observed during the establishment period of perennial rye grass crops in Oregon, USA (Mueller-Warrant *et al.,* 2014). Correlations were strongest in the two sites in which highest slug densities (mean trap counts of 9.8 and 21.1) were detected using surface refuge traps (accounting for 40-50 % of the damage). In comparison, sites with intermediate numbers of slugs displayed periodic (inconsistent) correlations, and no relationships were detected at those with low slug densities (mean < 3 slugs per trap). Irregular shaped patches of slug damage (approx. 50-150 m^2) occurred in the field that suffered the highest crop losses (Mueller-Warrant *et al.,* 2014). The weaker correlations may have resulted from behavioural responses to adverse weather conditions (e.g. high temperatures or prolonged periods of low rainfall). This can result in a large proportion of a slug population taking refuge in more protected environments beneath the soil surface or under a refuge, such as a stone, where they are difficult to detect using surface traps (South, 1992). Thus crop damage is likely to be an unreliable approach for slug patch identification, as confirmed in a recent study in winter wheat (Forbes *et al*., 2017).

1.3. Legislation affecting molluscicide use

1.3.1. Pesticide Authorisation Directive

Current EU and UK policy aims include optimising pesticide use in crop production to protect the environment and human health whilst maintaining agricultural efficiency. The Pesticides Authorisation Directive (PAD) (Council Directive 91/414/EEC) was introduced in 1993 and since its introduction there was a 50 % decrease in registered pesticides available for use between 1993 and 2012 (Carlile, 2006; Hillocks, 2012). Several other EU directives and regulations also govern the registration and use of pesticides, including Water Framework Directive (Council Directive 2000/60/EC), Sustainable Use Directive (Council Directive 2009/128/EC), Groundwater Directive (Council Directive 2006/118/EC), Regulation (EC) No 1107/2009 (approval of products), Regulation (EC) No. 396/2005 (maximum permitted levels in food), Regulation (EC) No. 1272/2008 (classification, labelling and packaging of pesticides) and Regulation (EC) 1185/2009 (statistics on PPPs). Whilst the focus of legislation is on health and the environment, it is important that the decrease in available pesticides does not have damaging consequences for farming productivity (The Andersons Centre, 2014).

1.3.2. Water Framework Directive

The Water Framework Directive (WFD) (Council Directive 2000/60/EC) is focused on improving the quality of water with the objective of ensuring all water bodies had ‘good’ ecological and chemical status by 2015. Of the 10,379 water bodies assessed in the UK in 2015, however, only 35 % were found to be of ‘good’ or ‘high’ ecological status, against the criteria set out in Annex V of the WFD (Council Directive 2000/60/EC). In addition, the EU Drinking Water Directive sets the maximum permissible level of any pesticide in drinking water at 0.1 $\mu g\ L^{-1}$ (Council Directive 98/83/EC). In order to achieve this objective continuous improvement to pest management procedures are required to ensure that any overuse of PPPs is avoided.

1.3.3. Sustainable Use Directive

More recently, EU Directive 2009/128/EC (Sustainable Use Directive) requires each member country to establish a National Action Plan for pesticide reduction, the protection of water courses and promotion of low input regimes through Integrated Pest

Management (IPM) (Council Directive 2009/128/EC). It is implemented in the UK by the resulting UK Plant Protection Products (Sustainable Use) Regulations (2012). The cumulative effect of such legislative action, combined with the cost of product development and registration, has led to fewer PPPs being available.

1.3.4. UK policy

There are regulations and acts which transpose the EU directives and regulations into UK law such as UK Plant Protection Products (Sustainable Use) regulations 2012 and a legally binding code of practice for using plant protection products (Defra, 2006), In response to the Sustainable Use Directive (2009/128/EC), Water Framework Directive (Council Directive 2000/60/EC), Groundwater Directive (Council Directive 2006/118/EC) and Drinking Water Directive (Council Directive 98/83/EC) the UK has adopted a voluntary approach to achieving implementation of legislation, with the Voluntary Initiative launched by the UK government in 2001 (Voluntary Initiative Steering Group, 2002). The aim of the Voluntary Initiative is to work with stakeholders to promote working practices that contribute to reducing the impact of farming on the environment, it also incorporates a National Register of Sprayer Operators (NRoSO), which during the first five years following its launch achieved a level of 88 % of arable land being sprayed by members of the NRoSO. The scheme ensures registered operators are kept up to date with legislation changes and best practice for applying chemicals (Glass *et al.,* 2006). As new directives have been implemented the Voluntary Initiative has evolved, for example, asking farmers to complete Integrated Pest Management Plans (IPMPs; replacing Crop Protection Management Plans) thus moving the focus to an integrated approach to tackling environmental impacts. The impact of the voluntary approach on plant protection product (PPP) retention remains to be established but future advances relating to molluscicide may be dependent, in part, on improved knowledge of slug behaviour and ecology (Forbes *et al*., 2017).

1.3.5. Potential effect of pesticide removal from market

In 2014 the Agricultural Industries Confederation, the Crop Protection Association and the National Farmers Union commissioned a review conducted by The Andersons Centre (research consultants) investigating the impact of current EU and UK legislation on the future availability of pesticides. The resultant report categorised all the remaining PPPs into high, medium and low risk of removal from the market, and showed that in the UK 35 % of remaining PPPs are at medium or high risk of loss (The Andersons Centre, 2014). Based on a scenario whereby only high-risk PPP’s (two out of the three active ingredients for slug control, (methiocarb and metaldehyde) were classed as at high risk of withdrawal) were lost annual yield losses due to slug damage were estimated at 3 % for winter wheat, 4 % for OSR and 2 % for potato crops (The Andersons Centre, 2014), making them one of the main pest groups in the UK (profit reduction based on 2016 yields and prices; Table 1.1.). Since the report was published both methiocarb and metaldehyde have or, in the latter case, likely will soon be removed from the market, which would leave ferric phosphate as the only active ingredient available for slug control (HSE, 2014; Appleby, 2019; Pickstone, 2019). In order to maintain sustainable crop production growers will need to consider targeted application methods which reduce the total amount of pesticide application whilst ensuring adequate levels of control.

Table 1.1. A comparison of the potential threat of different pests and diseases in wheat, oilseed rape and potato crops in a scenario where high risk PPP's are lost. Figures expressed as % yield loss (The Andersons Centre, 2014).

Pest/disease	Wheat	Oilseed rape	Potatoes
Slugs	3 %	4 %	2 %
Late blight (*Phytophthora infestans*)	-	-	10 %
Aphids/plant parasitic nematodes	2 %	2.5 %	2 %
Septoria tritici	3 %	-	-
Yellow rust	5 %	-	-
Volunteer cereals	-	24 %	1 %
Black grass	16 %	-	-
Turnip yellow virus	-	10 %	-
Cabbage stem flea beetle	-	7 %	-

1.3.6. Reduction in pesticide use

Increasingly EU policy is requiring growers to reduce the use of pesticides and investigate alternative options of control. For instance, the introduction of EU Directive 2009/128/EC requires National Action Plans for pesticide reduction to be established and the resulting UK Plant Protection Products (Sustainable Use) Regulations 2012 (PPP regulations) address the protection of watercourses and promotion of low input regimes, amongst other provisions (Defra, 2006). Cross compliance requires farmers to meet standards of good environmental practice (under Good Agriculture and Environmental Conditions and Statutory Management Requirements) in order to receive money from the basic payment scheme or from stewardship schemes (Defra, 2018a). This provides a financial incentive for farmers to meet the requirements of the PPP regulations and WFD. In recent years, metaldehyde has been detected in several water catchment areas at levels up to 8 $\mu g\ L^{-1}$ (Castle *et al.,* 2017). A metaldehyde stewardship group (MSG) was set up to mitigate the risk of removal due to the levels detected in water courses (AHDB, 2010).

Conflicting with the pressure to reduce pesticide usage is the demand for increased food production as the population grows. To feed the growing population an estimated 200 %

increase in global food production is needed by 2050 (Clay, 2011). To meet these demands alternative methods of control and application must be investigated.

1.4. Crop protection measures for slugs

1.4.1. History of slug management

Historically slugs have been managed through common farming practices including ploughing and crop rotations. Crop husbandry has changed over time, with many modern day farming practices being less beneficial in the reduction of slugs. For example, the prohibition of stubble burning (The Crop Residues (Burning) Regulations 1993), a move towards minimum tillage and direct drilling (Kennedy *et al.,* 2013), shorter rotations and larger areas of OSR grown mean there is more demand for effective slug control methods. Stubble burning aided the control of slugs by directly killing individuals and reducing the amount of food available on the soil surface (Glen and Symondson, 2003). The reduction in cultivations reduces the number of slugs killed by the mechanical action of the machinery (Port and Port, 1986; Kennedy *et al.,* 2013) and fewer slug eggs are exposed to the surface where they become desiccated (Glen and Symondson, 2003). Crops following OSR in rotation are more susceptible to damage from slugs, partly due to the dense canopy of the OSR crop, which provides ideal environmental conditions for increases in population size resulting in larger numbers being present during the often susceptible establishment period (Port and Port, 1986, Glen *et al.*, 1993). The timing of planting and rotations also affect damage levels; winter grown crops are more susceptible to slug damage as growth is slower, lengthening the time for which vulnerable stages are exposed to the pest (South, 1992). Of the range of commercial products available for slug control in arable crops, molluscicide pellets are the industry’s preferred approach. In 2016, 37 % of winter and spring sown wheat, 88 % of OSR and 100 % of potatoes were treated with a molluscicide in the UK (Garthwaite *et al*., 2018).

1.4.2. Molluscicide pellets; Methiocarb

For many years there have been at least two active ingredients (methiocarb and metaldehyde (discussed below)) concurrently available for the control of slugs. The primary method of application is in a bran based pellet containing the active ingredient, with the bran acting as an attractant to the slugs. Pellets can be broadcast across the field or mixed and drilled with the seed (South, 1992).

Methiocarb was one of most widely used active ingredient, especially in high value crops until it was removed from the market in September 2015 (HSE, 2014) due its detrimental effect on farmland birds (Clarke, 2014). Methiocarb is a carbamate, inhibiting acetylcholine esterase which causes paralysis (South, 1992). As well as the impact on farmland birds, negative effects have also been observed in other invertebrates (e.g.

Coleoptera). A 95 % reduction of carabid activity was detected following broadcast application and 85-90 % reduction when drilled (Purvis and Bannon, 1992).

1.4.3. Molluscicide pellets; Metaldehyde

Metaldehyde is a tetramer of acetaldehyde, which acts through contact or consumption by causing paralysis and excess mucus production (South, 1992). Metaldehyde has been the most widely used active ingredient for slug control, representing 75 % of the UK slug pellet market in 2016 (most recent figures; Garthwaite *et al.,* 2018), the implications of its likely removal from the market will be significant for slug management. The removal of metaldehyde from the market was announced in December 2018 (Defra, 2018b), in July 2019 an appeal meant the ruling was overturned on a technicality. Although the ban was lifted, it will likely be reinstated in the near future (Appleby, 2019; Pickstone, 2019), therefore for the purposes of this thesis it will be assumed that metaldehyde will not be available long term for slug control. Although metaldehyde has been detected in several water catchment areas at levels of up to 8 μgL^{-1} (Castle *et al.,* 2017), 80 times higher than the level permitted under the Drinking Water Directive (Council Directive 98/83/EC), there were well established stewardship guidelines and voluntary efforts to facilitate significant mitigation of the risk of contamination of water bodies. Stewardship guidelines include leaving 10 m margins around field boundaries and along water courses, maximum application and dose rates and recommendations for pellet application timings in relation to weather (MSG, 2019). The announcement of the removal of metaldehyde from the market in December 2018 was not a result of water contamination but due to the risk it posed to farmland wildlife (Defra, 2018b).

1.4.4. Molluscicide pellets; Ferric phosphate

Ferric phosphate is a relatively new product, approved for use in the UK since 2005 (AHDB, 2010). Ferric phosphate acts as a stomach poison, iron deposits in the digestive gland cause slugs to stop feeding and ultimately death (Triebskorn *et al.,* 1999). Field experiments conducted in Switzerland found ferric phosphate was less effective than other products in reducing the number of *D. reticulatum* in lettuce plots. The plots treated with ferric phosphate had significantly higher numbers of *D. reticulatum* than those treated with metaldehyde, with an average of 2.25 slugs per trap compared to 0.25 in the metaldehyde plots (Speiser and Kistler, 2002). Damage was significantly reduced in lettuce plots where ferric phosphate was applied when compared with untreated plots and there was no significant difference in damage between plots treated with metaldehyde or ferric phosphate in the lettuce crop (Speiser & Kistler, 2002).

Other experiments have shown that ferric phosphate can offer comparable levels of control to metaldehyde, reducing slug damage by more than 50 % (Evans and Barker, 2017). Field experiments conducted in potato crops in Lincolnshire and Edinburgh in 2015 and 2016 showed that ferric phosphate significantly reduced slug numbers compared to untreated controls and that three treatment applications were as effective as four over the growing season (Evans and Barker, 2017). There is evidence to suggest that ferric phosphate may have a detrimental effect on earthworms, with less activity and higher mortality observed in treatments containing ferric phosphate compared to metaldehyde and untreated controls (Langan and Shaw, 2006; Rae *et al.,* 2007), which could be reduced by targeting applications to those areas of fields with higher slug densities.

1.4.5. Integrated control

EU legislation requires pesticides to be used in conjunction with a range of management strategies such as biological, biotechnical, cultural, genetic and physical controls and at the minimum level required to keep pest populations below those that cause unacceptable economic losses (Council Directive 2009/128/EC). Alternative methods for control of slugs have been well documented (see sections 1.4.5.1 – 1.4.5.4), and may contribute to IPM strategies that reduce conventional pesticide usage.

1.4.5.1. Pathogenic nematodes

Specific nematode species, in particular *Phasmarhabditis hermaphrodita*, offer comparable slug suppression to metaldehyde in high value crops such as sprouts, lettuce and asparagus (Wilson *et al.*, 1995, Glen *et al.*, 2000, Iglesias *et al.*, 2003). *Phasmarhabditis hermaphrodita* enter under the mantle of the slug as dauer larvae, once inside the shell sac they develop into adults which reproduce until the slug dies. After the death of the slug they continue to feed and reproduce throughout the body of the slug until resources are exhausted (Wilson *et al*., 1993). The use of nematodes in cereals and oilseeds is limited due to the cost of application (nematodes (Nemaslug®): ~£110 per hectare; Metaldehyde: £10-15 per hectare; ferric phosphate: £17-24 per hectare (ADAS, 2010). The cost coupled with limited published evidence documenting efficacy (Rae *et al.*, 2007) and conflicting results (Iglesias *et al.,* 2003; Evans and Barker, 2017) has resulted in limited use in arable crops. Evans and Barker (2017) found that Nemaslug® did not effectively reduce slug damage in potato crops but could help reduce damage when used in conjunction with ferric phosphate. Iglesias *et al.* (2003) conducted studies in Spain between 1999 and 2001 to investigate the effect of the repeated use of molluscicides on non-target organisms. Treatments included metaldehyde pellets and four different nematode (Nemaslug®) treatments ((1) nematode application three days prior to planting, (2) slurry application seven days prior to planting, followed by nematode application three days prior to planting, (3) nematodes applied to the uncultivated area surrounding plots three days prior to planting and (4) nematodes applied three days prior to planting but only for the first crop) compared with an untreated control. Over the two-year experimental period crops (lettuce, leaf beet and cabbage) were planted as seedlings and harvested after 6 weeks. No significant differences were found between the numbers of slugs on harvested plants which had received treatments 2, 3 and 4 compared with untreated controls, however, there was a significant reduction in the damage observed to the crop (percentage leaf loss), following the metaldehyde treatment and nematode treatment (1) (Iglesias *et al.,* 2003).

Field experiments in winter wheat investigating application of different nematode dose applied either 1 or 4 days prior to planting (comparable treatment to treatment 1 in the work carried out by Igelsias *et al.,* (2003)) have found comparable control outcomes for *P. hermaphrodita* and methiocarb (Wilson *et al.,* 1995). Even if consistent and equivalent control levels to those achieved by ferric phosphate and metaldehyde were achieved in this crop, targeted approaches to nematode use would be still be required to reduce costs before commercial use became economically viable. Slugs are known to have a discontinuous distribution in arable fields (South, 1992; Bohan *et al.,* 2000a; Archard *et al.,* 2004; Mueller-Warrant *et al.*, 2014), so specific targeting of nematodes at the discrete patches of high slug numbers may reduce the amount of product used and promote wider uptake of nematode treatments in lower value crops.

1.4.5.2. Natural enemies

Toads, snakes, slow worms, birds and hedgehogs have all been found to predate slugs (South, 1992), however, invertebrates, particularly carabid beetles are recognised as being the main predatators of *D. reticulatum.* Although not sufficiently effective on their own, naturally occurring predators and parasites of slugs such as the carabid beetles *Pterostichus melanarius, Pterostichus madidus* and *Nebria brevicollis* may be used as components of an IPM strategy (Nash *et al.,* 2008). Laboratory experiments indicate ground beetle species vary in their potential as control agents and research investigating their impact on slug populations in the field should take account of the findings of Mair and Port (2001) who found that *P. madidus* and *N. brevilcollis* showed a preference for scavenging dead slugs over live slugs and only small slugs (<0.11g) were killed by either species. In particular, care must be taken when interpreting serological results from field studies as a high proportion of positive outcomes may result from scavenging of dead slugs rather than predation.

Carabid beetles are generalist predators and have the ability to utilise alternative prey when slug numbers are low. The frequent use of refuges below the soil surface when slugs encounter adverse environmental conditions and variations in slug populations between years may mean periods of low food availability for carabids. Switching to alternative prey during these times will reduce the impact on beetle numbers. Oberholzer and Frank (2003) investigated the feeding preferences of *P. melanarius* when presented with *D. reticulatum* alone and in combination with alternative prey. Alternative prey included live and dead crickets, live aphids and live dipteran larvae. Consumption of *D.*

reticulatum eggs was unaffected by the presence of any of the alternative prey offered. When beetles were offered live slugs on their own or in combination with alternative prey, despite no significant difference in the consumption of small/young slugs (average weight 0.033g), fewer medium and large slugs were consumed when offered in conjunction with dead crickets (Oberholzer and Frank, 2003). Symondson *et al.,* (2006) also found that the presence of alternative prey could impact the predation of slugs. In laboratory experiments there was significantly lower predation of slugs by *P. melanarius* when dipteran larvae were offered as an alternative prey. This effect was dependent on the combination of prey species available, for example no significant difference was recorded between the number of slugs remaining alive in the treatment group in which slugs were offered to the *P. melanarius* and the group in which slugs and earthworms were offered. The predation of slugs by carabids is supported by Ayre (2001), who found that in their experiments a large proportion of medium and large sized beetles predated one-day old slugs but that the level of predation at different temperatures varied between different species of carabid, reflecting the seasonal conditions during the peak activity period of each species. These results support the proposal that *P. melanarius* could contribute to the suppression of slug populations in the field, as consumption of eggs and juveniles of invertebrate pest species can significantly reduce population development.

The use of *P. melanarius* as a biocontrol agent was tested in a semi-field study comparing *D. reticulatum* populations in the presence of *P. melanarius* with varying levels of alternative prey (Oberholzer and Frank, 2003). Slug numbers in plots with the highest diversity of alternative prey and the largest numbers of beetles were lower than those without beetles, confirming the results of Symondson *et al.* (2006). Whilst *P. melanarius* has been shown to have potential as a useful control agent in experiments where they have been observed in a contained area, the predator-prey interaction is complex and requires further investigation. The importance of spatial and temporal coincidence of pest and predator in pest management systems was stressed by Schmitz and Barton (2014). Bohan *et al.* (2000b) mapped the spatial distribution of slugs (*A. intermedius* and *D. reticulatum*) and beetle populations (*P. melanarius*) in winter wheat and found that the distributions were temporally coincident, suggesting a direct association rather than opportunistic predation and supporting their potential use as a component of IPM strategies. *Nebria brevicollis* displays two activity peaks (late spring and autumn) which coincide with the peaks in reproduction of *D. reticulatum*, also enhancing their potential as a candidate biocontrol agent (Mair and Port, 2001). Further evidence of the predator-prey relationship between carabids and *D. reticulatum* is provided by the behavioural response of *D. reticulatum* to chemical cues. In an arena choice test, slugs avoided the zone on which *P. melanarius* had been maintained, with significantly more slugs found in the control zone after 24 hours. Slugs spent less time over the experimental period in the carabid exposed zone, moved faster and turned less frequently whilst in the exposed zone

(Armsworth *et al.,* 2005). Smaller slugs responded most quickly, supporting the findings of Ayre (2001) and Oberholzer and Frank (2003) who showed that slug predation is primarily due to predation of juvenile slugs.

1.4.5.3. Cultivation methods

Cultivation techniques such as ploughing may contribute to reduction of slug numbers by causing both direct physical damage and exposure to adverse environmental conditions, with a more significant effect in dry conditions resulting from increased desiccation of eggs (Glen and Symondson, 2003). Ploughing, however, can also have detrimental effects on other organisms, including carabids (Symondson *et al.,* 1996; Kromp, 1999). Conversely, zero tillage systems can help to maintain populations of predators such as carabids but may also lead to increased numbers of slugs, with the overall result of increasing damage. In a long term no-till field experiment analysis of the gut content of beetles showed that more slugs were being consumed by beetles and there were 1.8 times more beetles in the no-till plots when compared with the tilled (either ploughed or non-inversion tillage) plots. There was also a considerably higher slug biomass (x91) in the no-till plots (Symondson *et al.,* 1996). Seed bed preparation can also affect slug numbers; a fine, firm tilth reduces the number of cracks and refuges available to slugs. In heavier soils where this cannot be achieved, deeper drilling may make the seed less accessible to slugs reducing the damage to the crop caused by seed hollowing (Glen *et al.,* 1990b). Conflicting environmental, biological and practical impacts need to be balanced when considering the use of cultivation methods in IPM approaches to slug control.

1.4.5.4. Mixed cropping

Mixed cropping, providing alternative food sources can also reduce slug damage. For example, growing winter wheat in combination with selected wild plant species, such as *Taraxacum officinale* (common dandelion) and *Capsella bursa-pastoris* (shepherd's purse), resulted in reduced slug damage (Cook *et al.,* 1996). If an integrated approach includes providing an alternative food source, then this alternative must be consumed in preference to the crop, but must not negatively affect crop growth and should be available in sufficient quantity during the susceptible stage of the crop. Brooks *et al.* (2006) examined the use of *Trifolium pratense* (red clover) as an alternative food source and found that slugs consumed red clover in preference to wheat. In addition, mixed cropping had no significant effect on crop emergence and damage to the wheat seeds was reduced, making it a strong candidate for use in IPM strategies.

1.5. Techniques for estimating slug populations

There are several methods for assessing slug populations, some of which can be carried out in the field whereas others require samples to be returned to a laboratory. In this section soil washing, soil flooding, defined area traps (DAT) and refuge trapping will be reviewed, as the most common research methods, in relation to accuracy, efficiency and potential for use as a method of assessing slug populations in commercial settings.

1.5.1. Soil washing

Soil washing requires a known volume of soil to be returned to a laboratory where it is broken down using a water jet and 3 graduated sieves. The sieve and residue are put into a magnesium sulphate solution, where the organic matter floats to the surface and slugs and eggs can be removed (South, 1992). Soil washing is the most accurate sampling technique to determine absolute population numbers but its use for commercial assessments is limited as the method is too time consuming.

1.5.2. Soil flooding

Soil flooding is less destructive to the sample than soil washing. A known volume of soil is removed from the field and returned to the laboratory where it is placed in a container. Water is added to the container over a period of 4-5 days, forcing slugs to move to the surface of the sample where they can be removed and counted. Soil flooding gives comparable results (between 92 and 100 % of slugs being recovered) to soil washing for the number of juveniles and adults extracted, although eggs are not recovered (South, 1992). Although soil flooding is less laborious than soil washing the requirement for soil to be extracted and flooded over a period of several days would make it too time consuming and unsuitable for commercial use (South, 1992).

1.5.3. Defined area traps

Defined area traps provide a non-destructive method of assessing populations. In this method a barrier is placed around a known surface area of soil to a minimum depth of 10 cm, which prevents slugs moving in or out. As slugs appear on the soil surface they are removed until no further individuals are found. This technique is carried out in the field making it a less destructive method as well as saving time and labour. Under suitable environmental conditions i.e. mild and wet DATs can yield comparable results to soil flooding. For example, Ferguson and Hanks (1990) found no significant difference between the number of slugs found using the DAT and soil flooding techniques at four different field sites with different soil types. In less suitable environmental conditions the appearance of slugs on the surface may take longer as slugs are less active on the soil surface (Young *et al.,* 1993).

1.5.4. Refuge traps

Refuge trapping is a non-destructive method of assessing slugs, which involves placing a artificial refuge on the ground under which slugs congregate, typically an upturned plant pot saucer, a board covered in plastic wrapping or inverted turf (South, 1992). Refuge traps allow slugs to move freely in and out, providing an estimate of surface activity rather than an exact assessment of population density. Refuge traps are simple to use, and can be useful to compare levels of slug activity. Unfortunately, they do not provide absolute population sizes as slugs entering the traps may have travelled varying distances and a variable proportion of the population will be located below the soil surface and therefore not assessable by surface refuge traps (Clements and Murray, 1991; South, 1992). In addition, the number of slugs active on the soil surface will vary according to environmental conditions and time of day (Hommay *et al.,* 2003). In comparison to other methods of assessing slug populations there is evidence that refuge traps may overestimate slug numbers, with a higher number of slugs found in open refuge traps compared to enclosed surface traps (Glen *et al.,* 2003). There is conflicting evidence as to whether refuge traps underestimate the number of smaller slugs in a population. Archard *et al.* (2004) found the number of small slugs in traps were underestimated by refuge traps, whilst others such as Howlett *et al.* (2005) found no significant difference between the effectiveness in trapping large and small slugs. Although there are limitations for assessing absolute slug populations using refuge traps there is evidence that they can be used successfully to determine relative numbers of slugs (South, 1992). Research aimed at establishing which environmental factors determine patch location would benefit from a technique enabling slug dispersal below the soil surface to be tracked to enable an understanding of whether the slugs are remaining in the same area of the field when they are not visible above ground.

1.6. Experimental techniques - tracking slugs

Previous studies of *D. reticulatum* behaviour have attempted to track the movement of individuals using freeze-marking (Richter, 1976), dye injected into the slug (Hogan and Steele, 1986), UV dye (Foltan and Konvicka, 2008) and radioactive isotopes (Hakvoort and Schmidt, 2002). A common problem with these methods is the requirement for the slug to be on the soil surface in order to be located and be identified. In addition, the markers can be short-lived or difficult to distinguish in the field. For example, the radioactive isotopes used by Hakvoort and Schmidt (2002) could be identified for approximately 10 days after the radioactive feed source was removed whereas the freeze-marks used on the slug’s mantle by Richer (1976) only lasted for up to 2 months on mature *D. reticulatum* and juveniles had to be rebranded several times a year. Additionally, injected dyes used by Hogan and Steele (1986) were difficult to detect on darker individuals making it difficult to distinguish the markings in the field. Finally, although the use of radioactive isotopes is useful for population studies, it does not allow the identification of individual slugs.

1.7. Radio Frequency Identification Technology

1.7.1. Using Radio Frequency Identification tags to track slugs

Although radio frequency identification (RFID) technology has been used since the 1940's advances in the early 2000's led to increasingly small tags becoming available. In passive RFID tags an internal antenna converts energy from a reader that powers the chip, which then sends back a unique signal (code) to the reader allowing for identification of the unique tag (Want, 2006).

Grimm (1996) demonstrated that an RFID tag injected into the foot of *A. lusitanicus* slugs could be used to identify individuals in the field. The technique has only previously been tested using a relatively large species (e.g. *A. lusitanicus* <13 cm long) when compared with *D. reticulatum* (<5 cm). Following tag insertion, survival and egg laying (number of batches, number of eggs/batch) of *A. lusitanicus* were found to be unaffected, but consequences for feeding or locomotor behaviour were not investigated. The technique developed by Grimm (1996) has since been used successfully by Ryser *et al.* (2011) to assess survival rates of *A. lusitanicus* and *A. rufus* in the field. Other than egg laying, no assays investigating the sub-lethal effects of tag insertion on either species were carried out, but post-tagging/post-release recovery was comparable and it was concluded that observed variation in survival probabilities were due to species-specific differences. The method of tag insertion has since been used by Knop *et al.* (2013) to investigate the dispersal of *A. lusitanicus* and *A. rufus* in arable fields, demonstrating that the invasive *A. lusitanicus* had higher locomotor activity than the native species.

Although these studies show RFID technology has the potential to be used in investigations of the behaviour of individual *D. reticulatum*, further work is required to establish the sub-lethal effects of tag insertion on the survival and behaviour of the smaller species.

1.7.2. Individual based modelling of slug movement

Individual Based Modelling (IBM) can be utilised to investigate and understand the mechanisms underpinning patch formation and stability (Grimm, 1999) but relies on data describing slug dispersion behaviour within the soil horizon (classical modelling techniques focusing on overall population density/dynamics (Uchmanski and Grimm 1996)). IBM has been used successfully to identify driving variables underpinning practical conservation decisions in the agricultural environment. For example, landscape diversity but not the arrangement of habitats, was found to be crucial for long term survival of agrobiont linyphiid spiders in agricultural fields. In this case, increased number of refugia were found to support larger populations, whereas the tillage frequency was not an important factor (Thorbeck and Topping, 2005).

1.8. Precision agriculture

1.8.1. Current uses of precision applications

Precision agriculture is increasingly considered as a means of overcoming the issue of reducing inputs whilst maintaining production levels. Uniform applications of products such as pesticides and fertilisers across fields can result in inputs being applied to areas of the crop which do not require treatment. As many of the products applied to crops may have a negative impact on the environment if over-used, approaches that reduce the quantity applied may provide environmental benefits as well as reducing costs. Remote sensing using drones or sensors mounted on machinery can be used to detect disease (Mahlein, 2016), weeds, pests, soil nutrients and moisture (Liaghat and Balasundram, 2010) allowing specific areas of fields to be identified for targeting of application of inputs. This is rapidly evolving area of research has potential to enable the earlier detection of issues in crops (Khanal *et al.,* 2017). For example, global positioning systems (GPS) are used in conjunction with variable rate technology to apply product to the field at the rate required at the specific location according to disease, weed, pest and nutrient levels. Variable application of fertiliser and pre-emergence herbicide application using GPS bout-matching (AHDB, 2009) can be cost saving and environmental benefits have been demonstrated.

1.8.2. Potential for use in slug control

There is evidence that slugs are not uniformly distributed across arable fields (discussed in section 1.1.8.) and that the distribution may be influenced by soil characteristics (outlined in section 1.1.7). If a method could be developed for identifying the location of areas with higher slug densities, for example using a combination of soil characteristics, then maps of fields may be produced and used to identify areas of the field at high risk from slug damage. These areas could then be targeted with slug pellets using a variable rate applicator reducing the amount of pesticide required.

This thesis aims to improve understanding of the behaviour of *D. reticulatum* which underlies the discontinuous distributions (resulting in patches of higher densities) observed in arable crops, the temporal and spatial stability of the patches and whether their location can be determined using either crop damage or physical characteristics of the soil. If a sufficiently accurate method of predicting patch location can be established then application of control measures might be targeted in response to an appropriate action threshold without the requirement for trapping or direct assessment of slug numbers across the whole field.

The work has been conducted in four stages. First, the design of a standard assessment grid was investigated and a suitable distance between traps to enable detection of higher density slug patches established (Chapter 2). This standard grid was subsequently used in all field work investigating slug patch stability and their relationship with crop damage and soil characteristics.

The work reported in Chapter 3 utilised the standard grid to establish the presence of patches in commercial fields located in major UK crop growing regions, and their spatial and temporal stability throughout single growing seasons and between consecutive seasons.

Building on these findings, later Chapters (4 and 5) examine the relationship between slug patches, crop damage (Chapter 4) and soil characteristics (Chapter 5). Work on soil characteristics in Chapter 5 employed both laboratory experiments and field sampling to identify factors that may determine the location of higher density patches. The objective of the work described in these chapters was to indicate a sub-set of soil characteristics which will be the subject of future work to determine whether individually or in combination they can accurately identify where patches will exist in the field.

Finally, a technique is developed in Chapter 6 which uses RFID technology to track the movement of *D. reticulatum* both above and below the soil surface in order to investigate behavioural mechanisms that may underpin patch cohesion and stability.

Specific objectives and hypotheses will be defined in the relevant chapter.

Chapter 2. General Methodology

Methodology that is common to several aspects of the work reported in this thesis is described in this chapter. A method which facilitated the identification of areas with higher slug densities and allowed the temporal stability of these areas to be investigated was required. The trapping options and different inter-trapping distances are discussed. The resulting methodology will be used to investigate the distribution of slugs in arable fields but also to map the damage to crops caused by slugs and the variation of soil characteristics across the commercial fields in subsequent work. Methods which are unique to specific aspects of this research are described in detail in the chapter to which they are relevant.

2.1. Establishing a trapping method for assessing the distribution of slug populations in an arable field.

Several methods which have been widely used for assessing slug populations are discussed in the literature review (section 1.5.). For the purposes of this study, the trapping method adopted must enable the accurate identification of the location and dimensions of discrete patches with higher slug densities whilst allowing for temporal variation in slug activity and distribution above and below the soil surface, sample and map a sufficiently large area of the field to facilitate comparisons between the various characteristics assessed in relation to these patches, and allow any slug population movement across the field to be determined. A key component of this study involved the investigation of the temporal stability of slug populations and their distribution within arable fields. To reduce the risk of the trapping technique affecting the size of populations following a series of assessments taken in a restricted area of a field crop, a non-destructive sampling method was sought. Accordingly, DATs and refuge traps (see section 1.5.) were considered to be suitable candidates (Ferguson *et al.,* 1990; Archard *et al.,* 2004; Howlett *et al.,* 2005).

2.1.1. Comparison of defined area traps and refuge traps.

Defined area traps and refuge traps were compared in field tests during November 2015 to determine which method would allow the objectives of this thesis to be met most efficiently. Defined area traps and refuge traps were set in pairs (within 10 cm of each other) at the nodes of a 2 x 4 trapping grid with 20 m between adjacent nodes. The experiment was conducted at a field site South West of Harper Adams University (52° 45' 55.5732'' N 2° 26' 33.1728'' W) with clay loam soil (Cranfield University, 2019), containing a winter wheat crop. The minimum distance from a trap to the field margin was 50 m. Defined area traps consisted of a metal ring (21 cm diameter, 15 cm high) inserted into the ground to a depth of 10 cm, with a 14.5 cm diameter, 0.5 cm thick circular rubber mat placed inside the ring. On each assessment date, the number of slugs recovered from the soil surface inside the ring or on the rubber mat were recorded. Refuge traps, similar to those used by Glen *et al.* (1993), consisted of upturned 18 cm diameter plastic terracotta plant pot saucers (LBS Horticulture Supplies, Lancashire, UK). Saucers were chosen instead of mat traps as there is evidence that they are more efficient for use with *D. reticulatum* (Young, 1990). Refuge traps were checked on the same assessment dates as the DATs and any slugs found within the saucer or on the soil surface immediately below the saucer were recorded. Slug counts from both traps were carried out simultaneously, on seven occasions during a 15-day period (Table 2.1).

Meteorological data were collected from the Harper Adams University weather station (52° 46' 39.7056'' N 2° 25' 39.8928'' W), situated a linear distance of 1.7 km from the field site.

2.1.1.1. Data analysis

All statistical analysis was carried out using R Version 3.4.2. (R Core Team, 2013).

Tests for normality (using Shapiro-Wilk test) and equal variance (using Levene test) indicated that the data were not normally distributed and did not have equal variance and accordingly a Mann Whitney U test was used to compare the two trapping methods.

2.1.2. Results from the comparison of trapping methods

During the whole assessment period only four slugs were recorded in the DATs compared with a total of 21 slugs in the refuge traps. Significantly higher numbers of slugs were consistently recorded in refuge traps when compared with DATs throughout the experiment ($W = 1117$, $p = 0.00014$; Table 2.1). The weather conditions recorded during the trapping period (Table 2.2) were unlikely to have significantly deterred surface activity see (section 1.1.5.), facilitating the comparison of the two trap designs.

Table 2.1. Total slug counts from adjacent refuge traps (R) and Defined area traps (D), at 20 metre intervals in a 2 by 4 grid over a two-week period.

Date		11-11-15	12-11-15	16-11-15	18-11-15	20-11-15	23-11-15	25-11-15
Trap type	R	1	1	4	5	3	2	5
	D	0	0	1	0	2	0	1

Table 2.2. Weather data for trapping period 11/11/15 to 25/11/15.

Date	Minimum temperature °C	Precipitation mm
11/11/2015	13.1	0.0
12/11/2015	7.9	3.4
13/11/2015	5.9	0.2
14/11/2015	4.7	4.2
15/11/2015	7.5	0.8
16/11/2015	9.2	2.4
17/11/2015	4.4	0.8
18/11/2015	8.3	3.0
19/11/2015	8.6	0.6
20/11/2015	4.3	3.4
21/11/2015	1.1	0.8
22/11/2015	-1.0	0.0
23/11/2015	-2.9	4.6
24/11/2015	-0.9	1.8
25/11/2015	5.8	4.4

2.1.3. Selection of trapping method

Two of the main factors determining the onset and level of slug activity are reported to be daylight and temperature (Wareing and Bailey, 1985; Hommay *et al.*,1998; Section 1.1.5). *Deroceras reticulatum* can remain active and cause crop damage under a wider range of temperatures than most other slug species. Feeding has been recorded at temperatures as low as 0.8°C, while other slug species have been found to be inactive below 5°C (Mellanby, 1961). Moisture is also widely acknowledged to affect the number of slugs active on the soil surface with fewer occurring under dry (or waterlogged) conditions (Choi *et al.,* 2004; Shirley *et al.,* 2001; Young *et al.*, 1991). Records of temperature and precipitation from a local weather station (Table 2.2) indicated that conditions remained suitable for slug activity during most of the period during which the experiment was conducted, facilitating comparisons of the two trapping methods. Consistently more slugs were recorded from refuge traps than DATs on every sampling occasion, suggesting that refuge traps might offer a suitable method for use in the wider study.

The research in this thesis investigates the discontinuous distribution of slugs in commercial fields, the location of areas of higher densities and the stability of these higher density patches. Considering the aims, the trapping method should allow the free movement of slugs (both above and below the soil surface) to reduce the impact of assessment on patch formation and stability. There has been limited research on slug movement in the field but it has been suggested they can move up to 1.5 m each night (Rollo and Wellington, 1981). Using DATs would restrict slug activity, increasing the potential for inaccurate assessment of patch location and stability. Refuge traps allow slugs to both enter and leave, a new refuge to be selected each night and disintegration or movement of patches across the field to be unhindered and monitored over time.

A benefit of refuge trap design for the current study is that slugs are not removed from the study area, allowing natural variation in slug population size throughout the study period. In the DAT, slugs are confined within the area defined by the trap, which may affect the rate or extent of changes in population size. DATs can be used to establish point estimates of population size within a defined volume of soil, but are less well suited to addressing the assessment of factors reliant on both population size and individual activity. One of the widely cited drawbacks of refuge traps is that they assess surface activity and are therefore a less accurate method of assessing populations (Clements and Murray, 1991). This study, however, requires an assessment method that incorporates both slug density and the impact of spatial dispersion, making refuge traps a suitable candidate.

Although DATs allow estimates of slug numbers to account for vertical movement, lateral movement is restricted. Refuge traps only assess activity on the soil surface but do not restrict lateral movement. Repeated assessments conducted over extended periods of time, however, will facilitate assessment of slugs sheltering in soil horizons when environmental conditions encourage a return to the surface, using refuge traps in a time-series trapping programme may allow the accurate assessment of both patch stability and location.

The criteria for assessing the advantages and disadvantages of methods used for slug trapping are typically based on their suitability for farmers i.e. ease of use, cost effectiveness etc. For the purposes of research, methods are required, that take account of key aspects of the biology and behaviour of slugs. Refuge trapping used in protocols which incorporate assessment of surface activity and population size over time, without removing slugs from the study area offer a technique that addresses some of the major constraints of the work reported in this thesis. The selected standard trap was therefore, unbaited refuge traps consisting of upturned terracotta plant pot saucer 18 cm diameter (LBS Horticulture Supplies, Lancashire, UK).

2.2. Optimal characteristics of a trapping grid for investigation of slug patches in arable fields

Determining an appropriate trapping frequency across fields is important if accurate assessment of the characteristics of the discontinuous distribution of slugs to be achieved (Clark and Evans, 1954). If a grid which is too fine or too coarse is used then populations can appear uniformly or randomly distributed and the ability to detect patches even if they exist is lost (Bohan *et al.,* 2000a). A method which allows sufficiently large areas of fields to be monitored to facilitate identification of crop areas which lie between and within patches of higher slug density. Additional consideration of the time required to sample each grid, on each assessment day, were essential to allow the slug distribution in a number of fields to be carried out at regular intervals.

2.2.1. Comparison of three trapping intervals

To establish the appropriate resolution of refuge traps for assessing slug patches three sampling grids were tested over a two-week period during November 2015. Refuge traps (unbaited upturned plant pot saucers, 18 cm diameter, as described in section 2.1.3.) were placed at regular intervals in a rectangular grid at a field site sown with winter wheat, 1.7 km South West of Harper Adams University (52° 45' 55.5732" N 2° 26' 33.1728" W). The three grids had different internode distances of 2.5 m, 10 m and 20 m, with a single refuge trap set at each node. Slug counts were carried out between 0830 and 1000 and the number of slugs in each refuge trap and the soil surface immediately below the trap were counted and recorded. Slugs were not removed from the traps. The data were used to construct heat maps to illustrate patch location and facilitate further analysis.

2.2.1.1. Statistical analysis

Data were tested for normality (Shapiro-Wilk test) and equal variance (Levene test). ANOVA was used to determine any differences in the average number of slugs across the different trapping intervals.

2.2.2. Results of comparison of three different trapping intervals

The average numbers of slugs found in each trap at the three internode intervals were not significantly different ($F=1.99$, d.f.=1,16, $p=0.177$). Although weather conditions were suitable for slug activity (Table 2.1) low numbers of slugs were detected on all assessment dates but despite these low numbers areas of higher slug densities were clearly visible as demonstrated by the heat maps constructed using data from counts taken on 11 November 2015 (Figure 2.1).

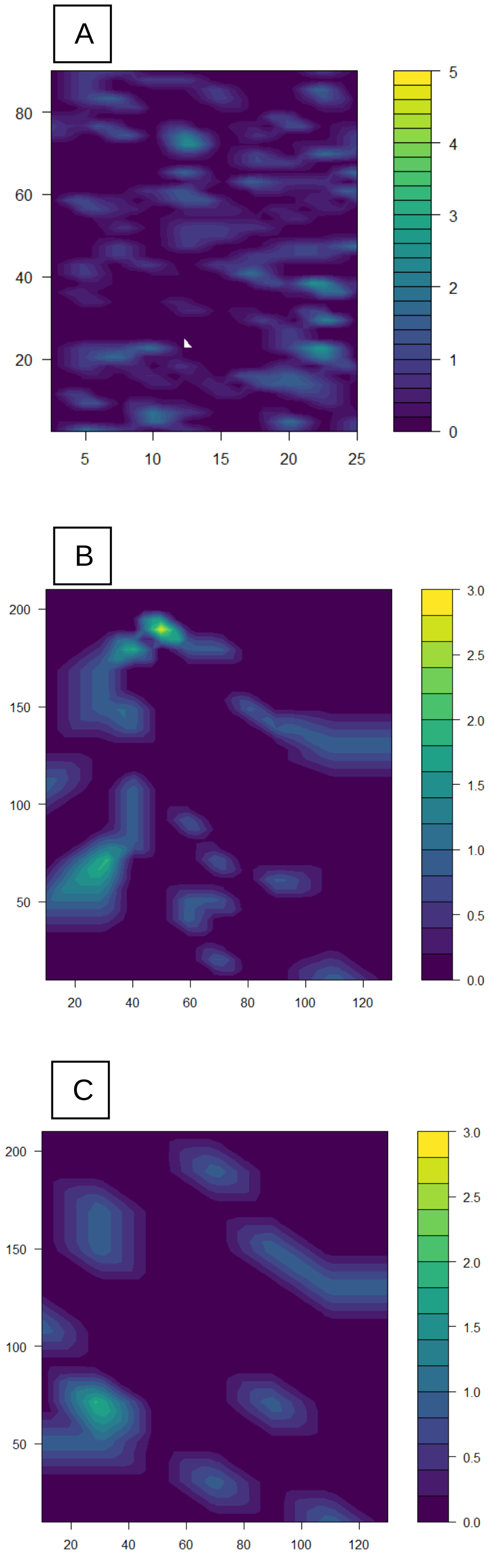


Figure 2.1. Map of slug distribution on 11-11-15 using a 2.5 metre minimum distance between traps (A), 10 metre minimum distance between traps (B) and 20 metre minimum distance between traps (C). X and Y axis scales show distance in metres and colours represent different numbers of slugs.

2.2.3. Design of standard trapping grid

The 2.5 m interval trapping grid required a large number of traps to sample a relatively small area of the field; a total of 1369 regularly spaced traps would be required to monitor the same area as 100 traps if an internode distance of 10 m was adopted. This resulted in assessments being labour intensive and reducing the number of replicate fields which could be sampled with the resources available, and so was eliminated from further analysis. The grids with internode distances of 10 and 20 m showed similar patterns of slug densities and patch location (Figure 2.1), but in the latter the patches were less well defined. The data collected were further analysed to investigate the optimum internode distance to employ in future work. Although conducted in collaboration with the author of this thesis (who is also a co-author of the published findings; Petrovskaya *et al*., 2018), the work was additional to the scope of the PhD programme and therefore is summarised here, and a copy of the published paper is provided in Appendix A.

The models developed during the analysis of Petrovskaya *et al.* (2018) predicted that that a coarser sampling grid (internode intervals of greater than 10 metre intervals) can be used to obtain accurate trap count estimates in fields with larger slug populations. In a field with low slug counts (Oadby 2016-17 season, see Table 3.1 and section 3.3.3.2.), however, reducing the 10 by 10 grid (10 m internode interval) to a 5 by 5 grid (20 metre internode interval) led to a lower average trap count, (1.07 slugs per trap reduced to 0.88; 18% error)). In this study the average difference between the number of slugs recorded in the 10 and 20 metre grids was 9.0%, varying between 1.9 and 17.9% on different assessment dates.

Petrovskaya *et al.* (2018) also found that a further reduction in the number of traps to a 3 x 3 grid (30 metre internode interval) and a 2 by 2 grid (40 metre internode interval) increased the error to 27 and 40% respectively. Although the errors incurred by using a coarser grid would be similar to those normally recorded in population monitoring (Meir and Fagan, 2000), a finer grid, which allowed the edges of patches to be more clearly defined was required for the purposes of this research.

To investigate the potential impact of individual traps (thus slug distribution at the time of sampling), a simulation was run in which the original grid was split into a number of equally sized rectangular sub-sections and the slug count from a randomly selected trap within each sub-section was used in the analysis. The simulation was then re-run using a different trap from each of the sub-sections, until all combinations of traps had been tested. The average trap count for 25 sub-sections (5 by 5 grid) was 1.09 with an average error of 12%, the maximum error being 24%. Similar simulations were carried out for 9 traps (3 by 3 grid), 4 traps (2 by 2 traps) and a single trap, demonstrating that as the number of traps decreased the error increased. For instance, the maximum error for 9 traps was 46%, whereas for 4 traps it was 69% and for 1 trap it was 144% (Petrovskaya *et al.,* 2018).

The models were recalculated using data collected under the current project from 100m x 100m grids established in three different locations (Adeney (Middle), South Kyme (1) and Uppington (1); see Table 3.1 and section 3.3.3.2.). This work suggests that to sample a 100 m x 100 m area of crop, a 5 by 5 grid of traps (25 traps in total) would give sufficient accuracy for both estimating slug populations and defining the location of higher density patches. For research purposes, however, to ensure that the most accurate information regarding slug patches and population estimates, can be collected using the available resources under this project, a 10 by 10 grid of traps was a more suitable option. The trapping design adopted for use the work reported in this thesis utilised a 10 by 10 grid with 10 metre internode intervals, and a single refuge trap of the design defined in section 2.1.3 set at each node.

Chapter 3. Identifying slug patches and their stability

This chapter uses the standard grid identified in Chapter 2 to investigate the distribution of slugs in arable fields. Here, the focus is on establishing the presence of patches, how stable they are over a growing season and between seasons. Later chapters will develop this to examine the relationship between these patches, damage and soil characteristics to investigate the potential for developing alternative methods for locating patches which don't require direct assessments of slug populations and facilitate targeted application of control measures.

3.1. Introduction

3.1.1. Spatial distribution

There are three possible distributions for a population in a natural environment, random, aggregated or uniform. As discussed in Chapter 2 an important consideration when establishing the appropriate sampling intensity for investigating a slug population distribution is the size and distribution of patches of higher slug numbers within a commercial field. Individuals may seem randomly distributed when a small specified area of a field is sampled, but when put into the context of a larger area (the whole field) the distribution may actually be aggregated (Clark and Evans, 1954). When sampling is conducted in the same environment over extended periods of time, patterns of distribution may change, for example alternating patterns of aggregated and random distributions of thrips (Rhodes *et al.,* 2011) and fruit flies (Papadopoulos *et al.,* 2003) have been observed in fruit orchards. Spatial distributions of populations have traditionally been explained as being a result of environmental heterogeneity and population growth (Taylor, 1984). The explanation for the temporal changes in distribution is not always clear and often requires further investigation of the underpinning biological and behavioural mechanisms determining the distribution, but can often be due to seasonal variation in the development stage of the insect, different reproduction/mortality rates in different areas, the dispersal behaviour of the insect or the distribution of hosts/refuges (Sciarretta and Trematerra, 2014).

3.1.2. Quantifying distribution patterns

When investigating the relationship between individual organisms within a defined area Clark and Evans (1954) devised a method of measuring the degree of randomness of a population. Calculating the expected mean distance between an individual and its nearest neighbour in a randomly distributed population, and sampling determined the actual (observed) distance, the ratio of observed to expected values can be determined, giving a measure (R) of randomness on a scale of 0 (maximum aggregation) to 1 (random distribution). More recently geostatistics have become a more common method of analysing spatial distribution data (Sciarretta and Trematerra, 2014), with semivariograms being the most common method, the semivariance of sample pairs is plotted against the distance between sampling points and a model is then fitted to best describe the spatial distribution. Two common sources of error when sampling populations over extended time periods are frequently encountered and should be avoided in future studies. Failure to take into account the rapidly changing population densities of some species due to rapid reproduction or population decline during the assessment period can result in false conclusions. Secondly, using only theoretical statistics without also including empirical ecological principles can also lead to false conclusions (Taylor, 1984). A primary requirement is that the known biology and behaviour of the species studied, and exploratory surveys, are used to determine the size, number and location of sampling points (Sciarretta and Trematerra, 2014). In the current study, pilot work was conducted (Chapter 2) that contributes to the establishment of reliable sampling protocols and monitoring outcomes.

3.1.3. Discontinuous distribution of slugs

The aggregation of *D. reticulatum* in arable fields is widely reported (South, 1992; Bohan *et al*., 2000a; Archard *et al*., 2004) with areas of high slug densities dispersed among areas of lower density. There is limited and conflicting research into the longevity of high density patches. Bohan *et al.,* (2000a) did not detect patch stability from a series of six assessments carried out between March 1997 and March 1998 in a winter wheat crop, whereas Mueller-Warrant *et al.* (2014) found stable patches in five grass fields when analysing between 8 and 15 assessments taken between October 2014 and February 2015. The differences observed in these two studies could be due to sampling method, (soil flooding compared with refuge traps).

Non-uniform distribution of slug populations may offer the potential for reducing molluscicide use in agricultural fields. If such patches are found to be sufficiently spatially and temporally stable, and a commercially viable method of identifying their location and dimensions can be established without the need for refuge trap counts then control measures may be targeted at high slug density patches alone, leaving areas with lower slug numbers untreated. Further investigation of this approach requires definite conclusions of the longevity and spatial stability of slug patches. If sufficiently stable various factors might be used to identify patch location including (but not limited to) crop damage or environmental factors such as soil moisture, soil pH or organic matter.

3.1.4. Current guidelines for pesticide application for slug control

Current guidelines for the application of slug control products recommend using refuge traps (upturned plant pot saucers) baited with chicken layers mash when the soil surface is moist and temperatures are between 5 and 25°C. In crops of up to 20 ha nine traps set in a 'W' shaped transect across the field are recommended (13 in larger fields). Traps should be left overnight and the number of slugs counted the following morning (AHDB, 2016). Established thresholds for OSR and wheat recommended that control measures are applied when a mean of 4 slugs per trap are recorded in a standing cereal crop (AHDB, 2016). The number of slugs active on the surface and so found in surface refuge traps varies widely according to weather conditions (Choi *et al.,* 2006; Hommay *et al.,* 1998; Willis *et al.,* 2008). Using the 'W' shaped transect, as recommended by AHDB (2016), may reduce inaccuracies associated with the discontinuous distribution of *D. reticulatum* resulting in more accurate assessment of slug populations (Petrovskaya *et al.,* 2012) but it does not distinguish sufficiently accurately between discrete areas of high and low slug densities to allow targeting of controls.

3.1.5. Current pressure on pesticide reduction

Increasingly farmers are facing pressure to reduce the amount of pesticide they use for the control of disease and pests (Hillocks, 2012). In regard to the control of slugs the two most widely used active ingredients until 2014 were metaldehyde (366 618 ha treated of the total 416 925 ha of wheat grown and 460 375 ha treated of the total 515 358 ha of OSR grown in 2014) and methiocarb (28 859 ha of wheat treated and 25 515 ha of OSR treated) (Garthwaite *et al.,* 2015). Ferric phosphate is a relatively new active ingredient, approved in 2005 and in 2014 it accounted for 21 379 ha of the 931 123 wheat and OSR area treated with molluscicides. In 2014 methiocarb was withdrawn for sale in Europe and distribution and permitted use of existing stocks ended in 2015 (HSE, 2014), as there was evidence it was detrimental to grain-eating farmland birds (Clarke, 2014). The withdrawal of methiocarb led to an increase in the usage of ferric phosphate, in 2016 13.7 % of molluscicide applications to wheat and OSR crops were ferric phosphate, an increase from 5.3 % in 2014 (Garthwaite *et al.,* 2015; Garthwaite *et al.,* 2018). Metaldehyde approval is also likely to be withdrawn imminently (Appleby, 2019; Pickstone, 2019), which would leave only one active ingredient, ferric phosphate, or more expensive alternatives such nematodes available for slug control (ADAS, 2010; Dörler *et al.,* 2019).

3.1.6. Measuring species aggregation

3.1.6.1. Nearest neighbour technique

Before a method for assessing patch location can be developed a definition of the characteristics of a patch is first required. Defining aggregation or patches has been a contentious issue for a long time (Taylor, 1984) as there is no commonly agreed definition of aggregation. Using trap counts from a grid of traps, such as that described in Chapter 2 means nearest neighbour techniques such as those described by Clark and Evans (1954) and Cook (1981) are redundant, and so will not be used in the current study.

3.1.6.2. Threshold value

Different options have been proposed for patch definition from trap counts. A crude method which includes all traps with a count above a threshold value as being part of a patch, regardless of the number of traps within each patch has been used in some studies but has proved to be relatively inaccurate when compared to other methods.

3.1.6.3. Mean count

An alternative approached has been suggested whereby the mean for the sampling area is calculated and if above the action threshold traps with the highest counts removed and allocated for treatment. The mean is then recalculated for the remaining traps and if it still exceeds the threshold the procedure is repeated until the last mean is below the recognised action threshold. This method could, however, leave traps in untreated areas outside of the patches with very high slug counts. An approach which uses the mean and variance could alleviate this issue. Taylor's power law a widely used index of aggregation which was originally defined to assess the spatial clustering of organisms in ecological systems (Cohen and Xu, 2015). It relates the variance of the number of individuals of a species per unit area of habitat to the corresponding mean by a power law relationship (Taylor, 1961). For a population mean size m and variance S^2, Taylor's law states that:

$$S^2 = am^b$$

Where a = constant; b = the index of aggregation estimated from the gradient of the line when the log variance is plotted against log mean

3.1.6.4. Comparison of distribution to random

Hotspot analysis can isolate areas where a population distribution is significantly different to that expected for a randomly distributed population. Individual counts within a sample are compared to the other values based on the deviance from a statistical distribution and the relative magnitude to other values within the data set. Individual data points, which are significantly higher than expected are identified (Darrouzet-Nardi, 2018). Taylor’s Power Law allows the aggregation of a species to be measured but there is no method of identifying which trap counts are contributing to the aggregation, therefore the index of aggregation will initially be used to confirm that the slugs are not randomly distributed and then Hotspot analysis will be used in this chapter to identify the trap counts which are significantly higher than if the distribution was random across the grid.

3.1.7. Commercial considerations

To develop a commercially realistic approach to targeting patches with controls some assumptions must be made. Firstly, when spreading molluscicides, the application equipment will be driven along tram lines, with pellets distributed onto the crop a standard distance from the centre point on either side. Thus, it can be assumed that applications are made to rectangular areas of land which contain (but are not necessarily fully covered by) one or more patches with higher slug numbers (Figure 3.1). The second assumption made here is that in order to achieve adequate control current threshold values are reliable, current advice in winter cereal or OSR crops suggest applying slug control when the mean number of slugs in 20 traps spread across the field is 4 or above (AHDB, 2016).

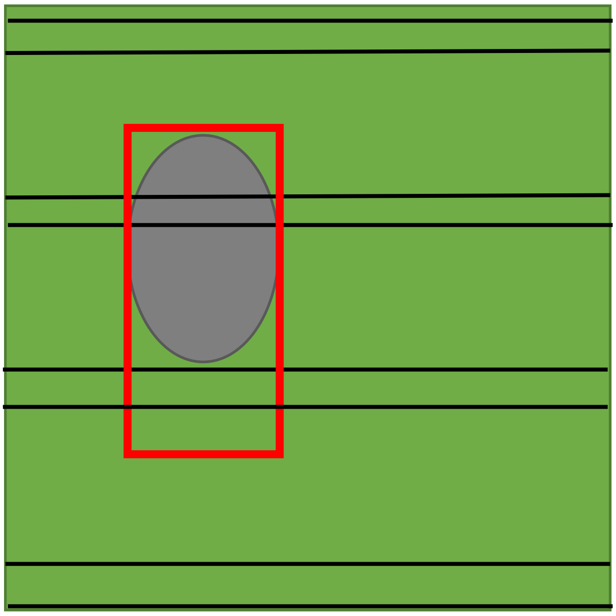


Figure 3.1. Application of slug pellets to a rectangular area of crop (inside the red line) containing irregular shaped high density slug patch (shaded grey), black lines represent tram lines.

The commercial feasibility of targeting application of control products to patches of higher numbers of slugs (potentially when current action thresholds are exceeded) relies on growers gaining either an advantage related to product stewardship/reduction in environmental impact, or cost saving. Advantages accrued in relation to either consideration relies on of the proportion of the field that would be treated. In this study this will be calculated from the rectangular treatment areas rather than proportion of the field covered by slug patches alone.

3.1.8. Objectives and hypotheses

The work reported in this chapter investigates patches of higher slug numbers occurring in commercial winter wheat or oilseed rape fields with the aim of investigating the spatial and temporal stability of areas of high slug densities in arable fields for the purpose of targeting the application of control measures.

Objectives

- To locate high density slug patches using the 10 x 10 trapping grid identified in Chapter 2.
- To determine how stable these areas are over time.
- To assess suitability of the size, number and stability of patches in relation to targeting application of molluscicides.

Hypotheses

- Clear areas with high slug densities can be identified and defined within arable fields in the UK.
- The areas of high slug densities identified are sufficiently spatially and temporally stable to facilitate the targeted application of controls.
- The individual and cumulative size of the slug patches would allow the efficient targeting of control treatments.

3.2 Materials and Methods

3.2.1. Field sites

During the first year of the study (2015-2016) experimental work was conducted at five field sites within close proximity of each other (maximum 17.4 km apart), and each with a similar crop rotation (fields 1-5; Table 3.1). In the second year of the study (2016-2017) fields were selected from a wider geographical area (incorporating eastern counties; Lincolnshire, Nottinghamshire and Leicestershire, as well as Shropshire and Lancashire) and different crop rotations (fields 2-13; Table 3.1). In the final year (2017-2018) a sub-set of these fields were studied, maintaining the geographical spread, with the addition of two new sites not previously sampled (fields 2, 6, 7, 14 and 15; Table 3.1) and facilitating comparisons in successive cropping years.

Table 3.1. Summary of field locations and crop rotations for field sites used during 2015-16, 2016-17 and 2017-18. In each case the crop grown prior to the commencement of experimental work is also reported. Crops two years prior to the commencement of assessments and post-assessment completion are not shown (shaded grey).

Field no.	County	Field	Crop 14- 15	Crop 15-16	Crop 16-17	Crop 17-18
1	Shropshire	Adeney (Corner)	oilseed rape	winter wheat		
2	Shropshire	Adeney (Middle)	oilseed rape	winter wheat	winter barley	oilseed rape
3	Shropshire	Lynn (Badjics)	oilseed rape	winter wheat	fallow	
4	Shropshire	Lynn (Stoney Lawn)	oilseed rape	winter wheat	fallow	
5	Shropshire	Uppington (1)	oilseed rape	winter wheat	fallow	
6	Leicestershire	Oadby		oilseed rape	winter wheat	cover crop
7	Lancashire	Wigan		oilseed rape	winter wheat	fallow
8	Lincolnshire	South Kyme (1)		oilseed rape	winter wheat	
9	Lincolnshire	South Kyme (2)		spring wheat	spring wheat	
10	Lincolnshire	Dog Dyke		winter wheat	winter wheat	
11	Leicestershire	Hoby		oilseed rape	winter wheat	
12	Nottinghamshire	Flawborough		oilseed rape	winter wheat	
13	Shropshire	Bridgnorth		winter wheat	oilseed rape	
14	Shropshire	Uppington (2)			oilseed rape	winter wheat
15	Lincolnshire	Belchford			spring beans	winter wheat

3.2.2. Trap counts

The 10 by 10 grid (10 m between nodes) described in section 2.2. of this thesis was used at all field sites to assess the number of active slugs on the soil surface. Grids were positioned within fields to include an area where the grower had historically found high numbers of slugs and to extend beyond to an area with historically fewer slugs (based on the grower’s knowledge of the field). The minimum distance of the grid from the field boundary was 20 m. Refuge traps (upturned terracotta plant pot saucers (LBS Horticulture Supplies, Lancashire, UK), 18 cm diameter) placed at each node of the grid were not baited (in order to not attract slugs to a food source). In 2015 – 16 slug assessments were carried out at 14-day intervals, with additional assessments at approximately 5-day intervals in two of the fields; Adeney (Middle) and Lynn (Stoney Lawn) between January and February 2016. In 2016 – 17 and 2017 – 18 slug assessments were carried out at approximately monthly intervals.

3.2.3. Slug classification

At each assessment, the slugs found in each refuge trap were identified (as *D. reticulatum*, *Arion* spp or *T. budapestensis*), counted and recorded under three size categories (large (>100 mg), small (<100 mg and >5 mg) or very small (<5 mg)). Slugs were counted *in situ* and were immediately released under the trap in which they were found.

3.2.4. Environmental conditions

Maximum and minimum air temperatures, soil temperature at 10 cm depth and rainfall were obtained from the Harper Adams University weather station, situated a minimum 1.6 km from the nearest (Adeney (Middle) and maximum of 15.3 km (Uppington (1) field site in Shropshire in all three field season, 2015-16, 2016-17 and 2017-18.

3.2.5. Statistical analysis of slug patches and stability within and between growing seasons

All statistical analysis was carried out in R Version 3.3.1. (R Core Team, 2013).

3.2.5.1. Slug counts

ANOVA was used to determine differences between slug counts in different fields and years. Post-hoc Tukey's test was used to determine where differences occurred.
Maps of slug counts created using the interp and filled.contour functions in R. The number of slugs in between traps was calculated by polynomial interpolation.

3.2.5.2. Hotspot analysis

The presence of hotspots was determined using the ScanLRTS function in R. The ScanLRTS function compares the observed number of individuals at each location on the grid with those expected if the population was randomly distributed. Areas where significantly higher counts than expected are identified are highlighted.

3.2.5.3. Taylors Power Law

Taylor's Power Law was used to calculate an index of aggregation during each growing season. The mean and variance for each assessment date were calculated and then the log of each was taken. The correlation between the log (mean) and log (variance) was calculated using Pearson's correlation coefficient (r) and was calculated for the assessments within each field season (2015-16, 2016-17 and 2017-18) as well as for the three field seasons combined. In each case the regression coefficient of the line of best fit was calculated, which equates to the index of aggregation.

3.2.5.4. Patch stability

The stability of patches was investigated using Pearson's Product Moment Correlation coefficient (r). Areas of higher slug densities were identified by locating the highest trap counts on each sampling occasion and where these occurred in the same area on more than 50 % of assessments the area was identified by a red box.

3.3. Results

3.3.1. Slug counts

The number of slugs detected varied between assessment dates within fields, between fields and years. There was a significant difference between the mean slug counts in each year (F= 41.74, d.f.=2, 157, p<0.001). The mean number of slugs at each field site was 451.3 (±63.8) in 2015-16, 55.5 (±7.0) in 2016-17 and 145.7 (±40.2) in 2017-18. There was also a significant difference between fields in all years, identified by a post-hoc Tukey's test, in 2015-16 Lynn (Stoney Lawn) had a significantly higher mean number of slugs than the other fields (F=12.10, d.f.=4,41, p<0.001), in 2016-17 the mean number of slugs in Dogdyke had significantly fewer slugs than Wigan and Uppington (1) and the mean number of slugs was significantly higher in Uppington (1) than Lynn (Badjics), Flawborough, Lynn (Stoney Lawn), Bridgnorth, South Kyme (2) and Adeney (Middle) (F=3.89, d.f.=311,77, p<0.001). In 2017-18, Uppington (2) had a significantly higher mean number of slugs compared to the other fields sampled (F=8.14, d.f.=4,27, p<0.001).

In general numbers of slugs were lower in assessments carried out in August, September and October and then, a small peak occurred between November and January followed by a larger peak in the spring (between March and May), where assessments continued after May numbers decreased (Figure 3.2). In 2015-16 assessments started in December, a peak in the spring was observed with a significantly higher number of slugs occurring in March (F=16.79, d.f.=5, 30, p<0.001; Figure 3.3). Although there appears to be some indication of the general trend for an autumn and spring in the 2016-17 and 2017-18 sampling periods (Figure 3.3), there were no significant differences in the number of slugs observed in different months. The number of slugs recorded in the 2016-17 and 2017-18 sampling periods were significantly lower (F=24.27, d.f.=2, 134, p<0.001).

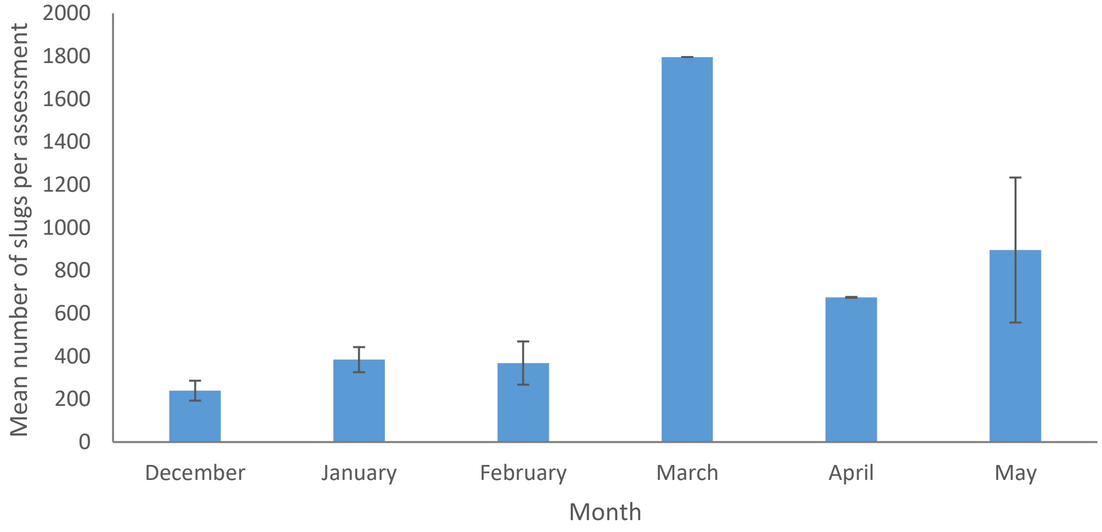


Figure 3.2. Mean number (±standard error) of slugs observed on each sampling visit in each month during the 2015-16 sampling period.

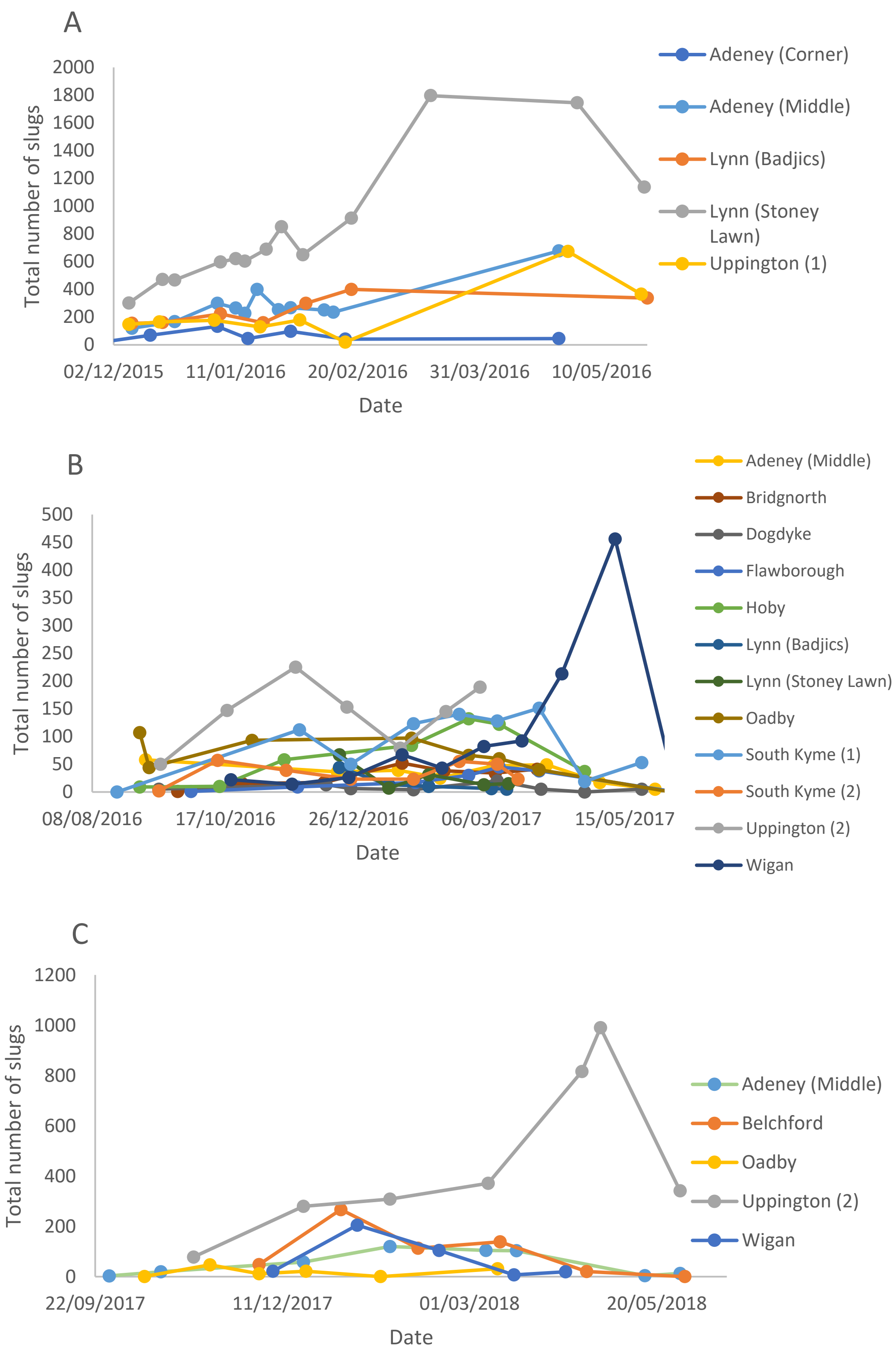


Figure 3.3. Variation in slug numbers during three winter wheat and oilseed rape growing seasons; (A) 2015-16, (B) 2016-17 and (C) 2017-18. Assessments represent the total number of slugs recorded in 100 refuge traps in each field; Adeney (Corner), Shropshire, Adeney (Middle), Shropshire, Lynn (Badjics), Shropshire, Belchford, Lincolnshire, Flawborough, Nottinghamshire, Hoby, Leicestershire, Oadby, Leicestershire, South Kyme (1), Lincolnshire, South Kyme (2), Lincolnshire, Uppington (1), Shropshire, Uppington (2), Shropshire and Wigan, Lancashire.

3.3.2. Slug aggregation

3.3.2.1. Hotspot analysis

Hotspot analysis was carried out for all sampling visits to all field sites over three seasons. Irrespective of the variation between the size of slug populations discrete areas of higher slug densities were observed in all fields (except on one occasion in each of Adeney (Middle) (August 2016), Belchford (June 2018), Dogdyke (April 2017), Hoby (September 2016), South Kyme (1) (August 2016) and Wigan (March 2018); Figure 3.4 - Figure 3.9; Table 3.2).

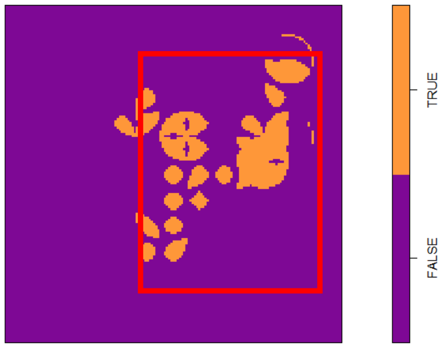
Table 3.2. The number of sampling occasions in each field and field season when hotspots (significant aggregations) were detected or undetected from slug counts across a 10 by 10 grid. Hotspot analysis identified areas where slug numbers were significantly ($p<0.05$) different to that expected if the population was uniformly distributed across the sampling grid.

Field	Field season	Number of occasions significant Hotspots detected	Number of occasions no significant Hotspots detected
Adeney (Corner)	2015-16	7	0
Adeney (Middle)	2015-16	11	0
Adeney(Middle)	2016-17	8	1
Adeney (Middle)	2017-18	8	0
Lynn (Badjics)	2015-16	7	0
Lynn (Badjics)	2016-17	5	0
Belchford	2017-18	5	1
Bridgnorth	2016-17	7	0
Dogdyke	2016-17	7	1
Flawborough	2016-17	7	0
Hoby	2016-17	6	1
Oadby	2016-17	8	0
Oadby	2017-18	5	0
South Kyme (1)	2016-17	8	1
South Kyme (2)	2016-17	8	0
Lynn (Stoney lawn)	2015-16	13	0
Lynn (Stoney lawn)	2016-17	5	0
Uppington (1)	2015-16	8	0
Uppington (1)	2016-17	7	0
Uppington (2)	2017-18	7	0
Wigan	2016-17	10	0
Wigan	2017-18	4	1

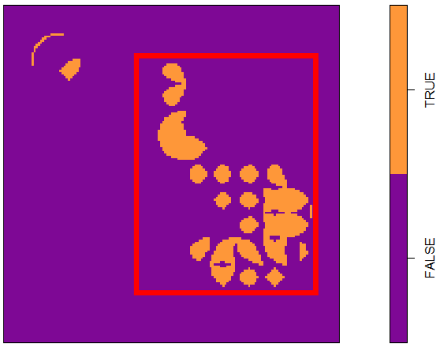
The results of the hotspot analysis for two fields in each of the three field seasons are summarised in Figure 3.4 to Figure 3.9. The hotspots (areas of significantly higher numbers of slugs than expected in a random distribution) in 2015-16 season in Adeney (Middle) appear in the same area of the field in 10 of the 11 assessments (Figure 3.4) and in Lynn (Badjics) on 7 out of 7 assessments (Figure 3.5). In 2016-17 the hotspots at Uppington (1) occur in three areas of the field, with those in the largest of the three areas occurring in all 7 assessments. Of the two smaller areas, one area contains a hotspot on 5 of the 7 assessments and the other 4 of the 7 assessments (Figure 3.6). In Wigan, hotspots occurred in the same area of the field on all 10 assessment dates (Figure 3.7). In 2017-18 the hotspots in Uppington (2), hotspots were detected on all assessment dates, occurring in the same area of the field on all 7 occassions (Figure 3.8). Although hotspot analysis indicated that in most fields in most years areas of higher slug densities consistently appeared in the same locations in the arable fields some exceptions occurred. For example, in Wigan (2017-18), hotspots were not consistently found in one area of the grid and on one assessment date no hotspots were present (Figure 3.9).

8-12-15

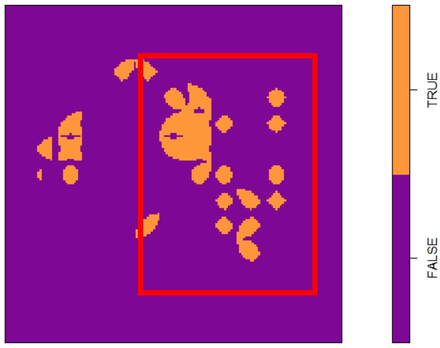
22-12-15



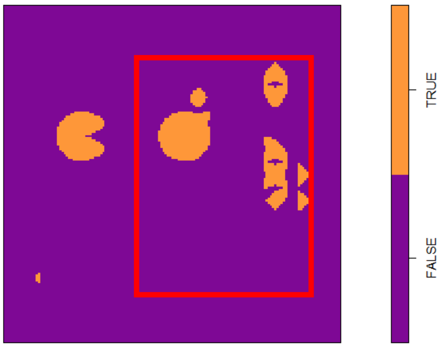
5-1-16



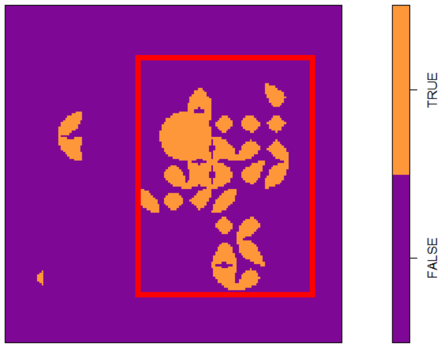
11-1-16



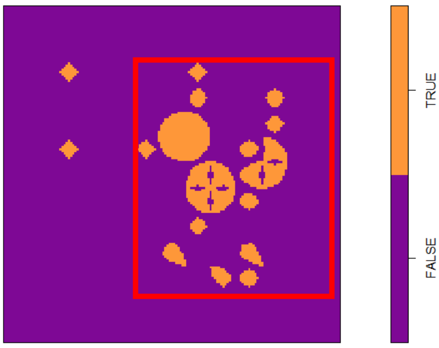
14-1-16



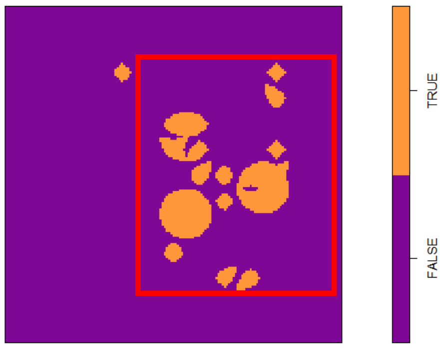
18-1-16



25-1-16



29-1-16



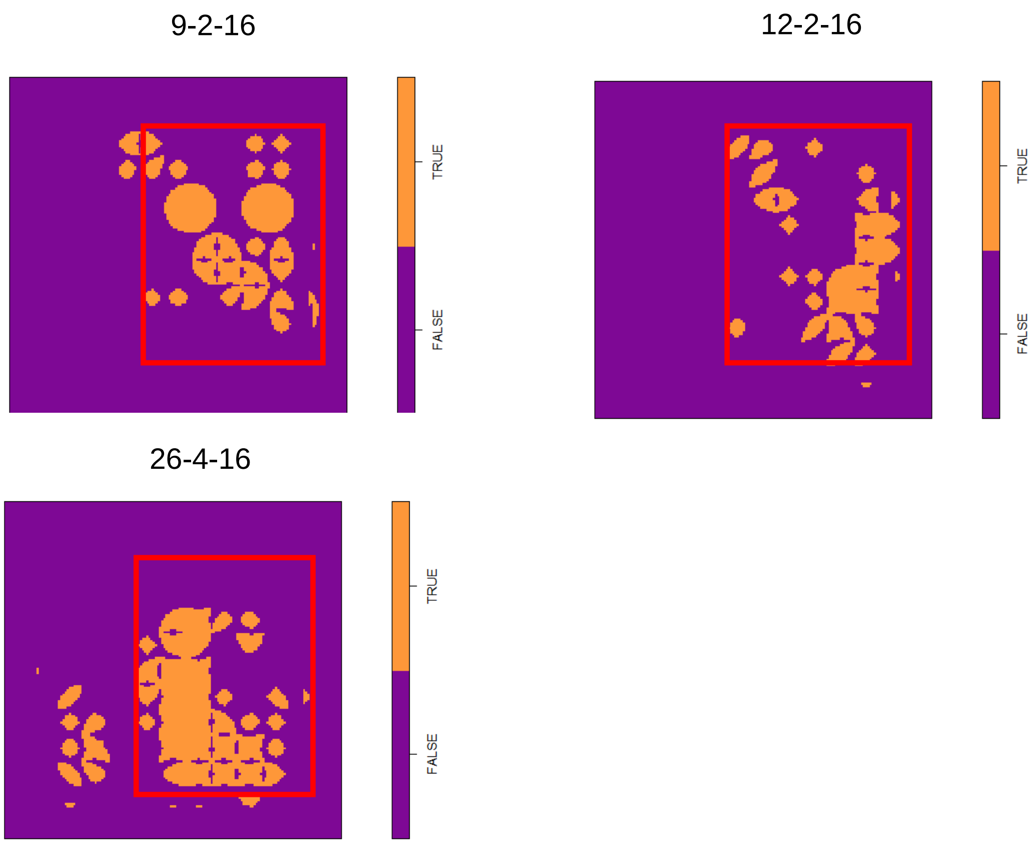


Figure 3.4. Hotspot analysis for Adeney (Middle) for assessment dates throughout the 2015-16 growing season. Orange represents significant aggregations of slugs, areas where more slugs were found than would be expected if the slugs were randomly distributed across the field at $p<0.05$ significance level. Purple shows the areas of the field where the number of slugs observed was not significantly different to that expected if they were randomly distributed. The red boxes highlight the areas where hotspots most frequently occur.

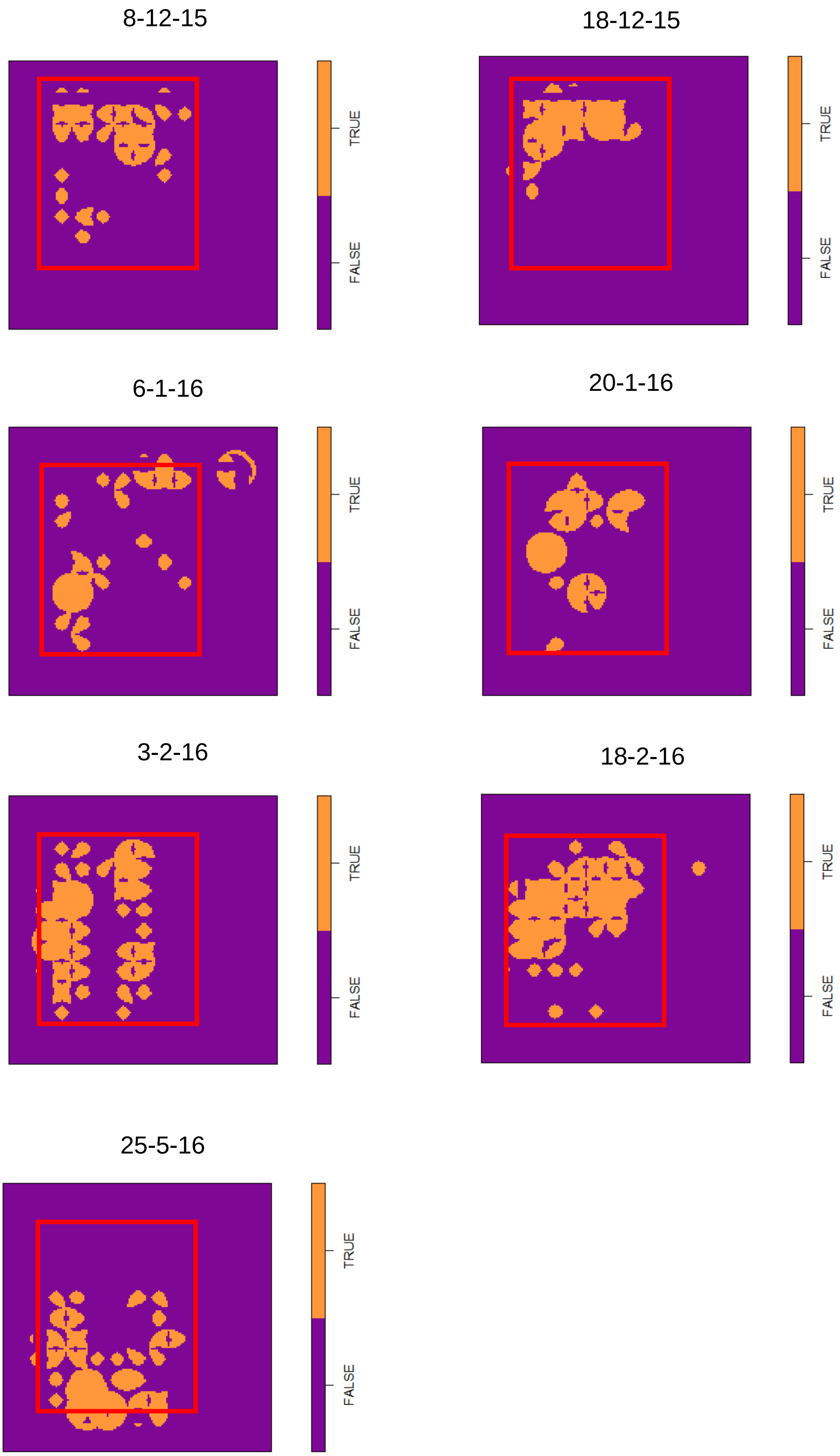


Figure 3.5. Hotspot analysis for Lynn (Badjics) for assessment dates throughout the 2015-16 growing season. Orange represents significant aggregations of slugs, areas where more slugs were found than would be expected if the slugs were randomly distributed across the field at $p<0.05$ significance level. Purple shows the areas of the field where the number of slugs observed was not significantly different to that expected if they were randomly distributed. The red boxes highlight the areas where hotspots most frequently occur.

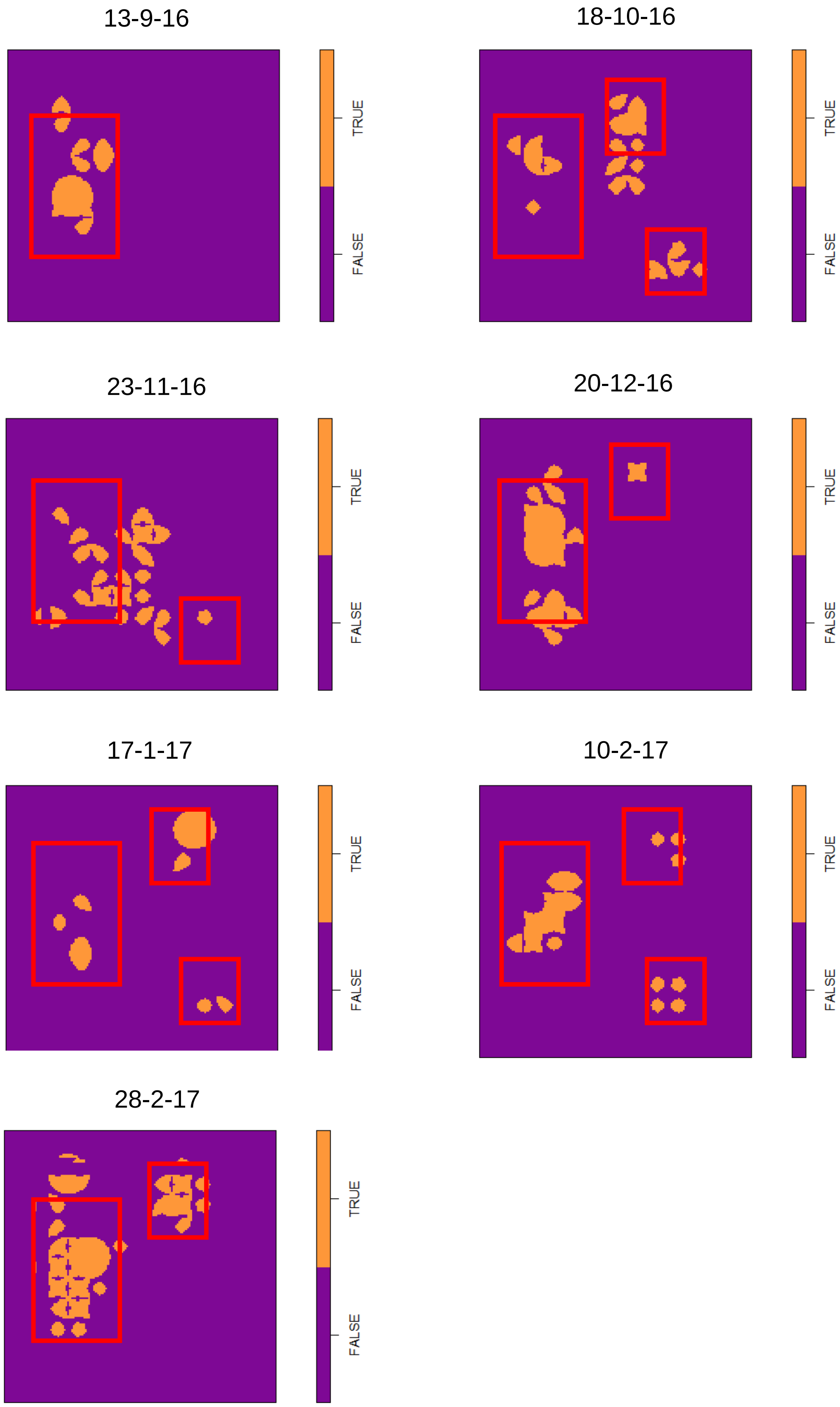
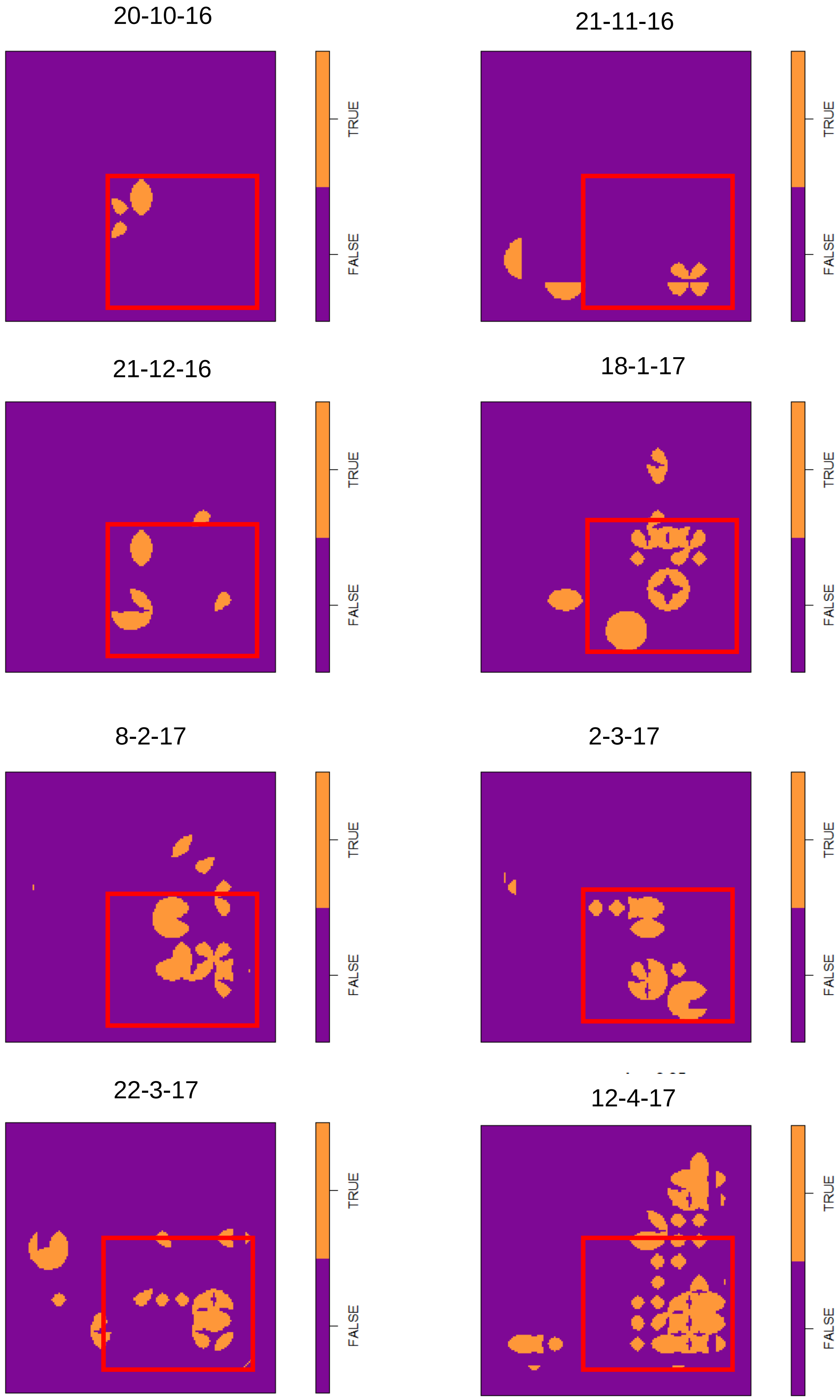


Figure 3.6. Hotspot analysis for Uppington (1) for assessment dates throughout the 2016-17 growing season. Orange represents significant aggregations of slugs, areas where more slugs were found than would be expected if the slugs were randomly distributed across the field at $p<0.05$ significance level. Purple shows the areas of the field where the number of slugs observed was not significantly different to that expected if they were randomly distributed. The red boxes highlight the areas where hotspots most frequently occur.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8-2-17
2-3-17
22-3-17
12-4-17
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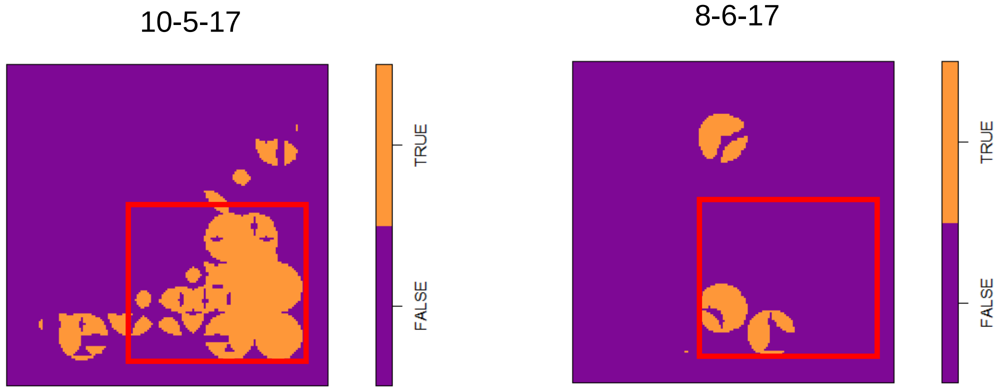


Figure 3.7. Hotspot analysis for Wigan for assessment dates throughout the 2016-17 growing season. Orange represents significant aggregations of slugs, areas where more slugs were found than would be expected if the slugs were randomly distributed across the field at $p<0.05$ significance level. Purple shows the areas of the field where the number of slugs observed was not significantly different to that expected if they were randomly distributed. The red boxes highlight the areas where hotspots most frequently occur.

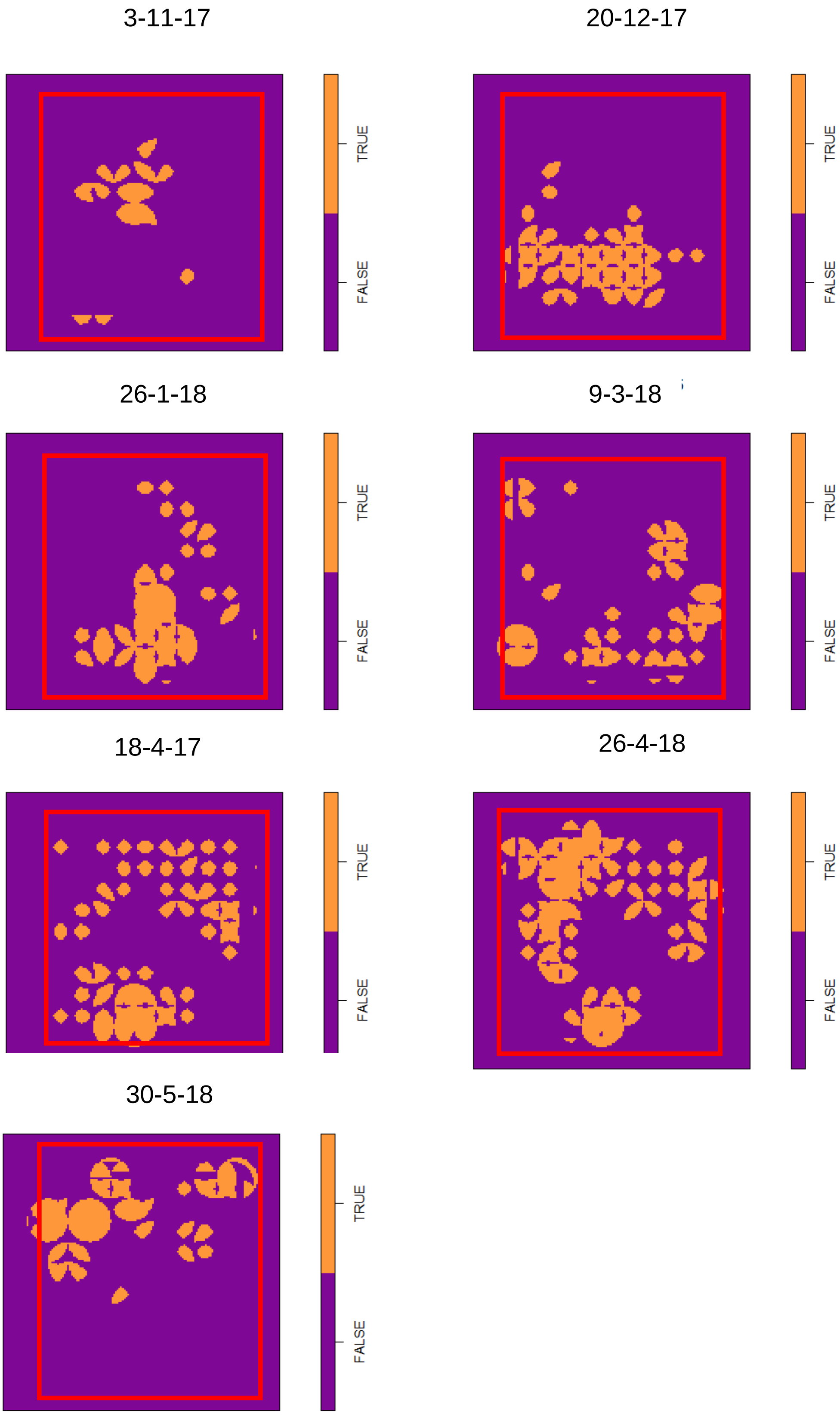


Figure 3.8. Hotspot analysis for Uppington (2) for assessment dates throughout the 2017-18 growing season. Orange represents significant aggregations of slugs, areas where more slugs were found than would be expected if the slugs were randomly distributed across the field at $p<0.05$ significance level. Purple shows the areas of the field where the number of slugs observed was not significantly different to that expected if they were randomly distributed. The red boxes highlight the areas where hotspots most frequently occur.

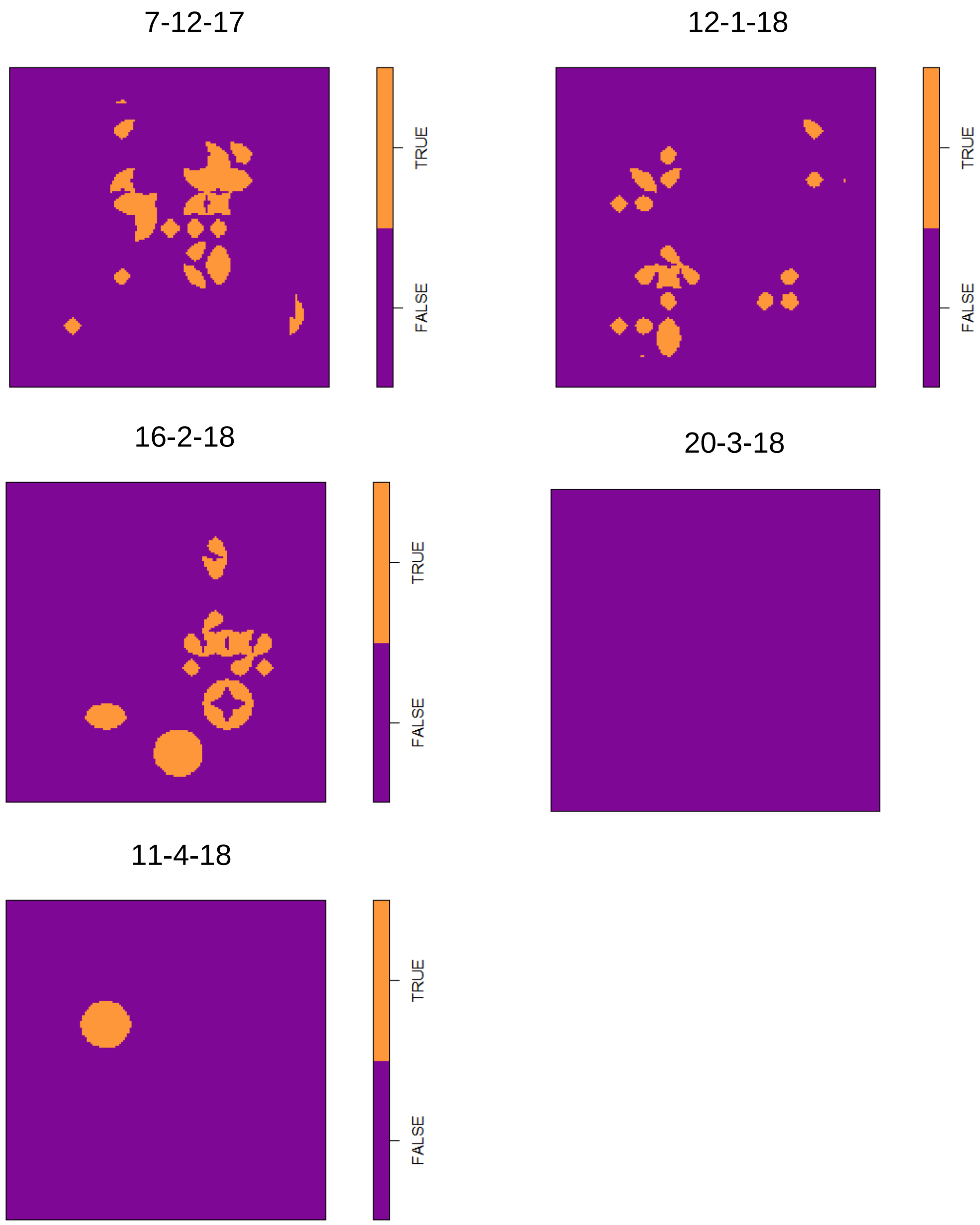


Figure 3.9. Hotspot analysis for Wigan for assessment dates throughout the 2017-18 growing season. Orange represents significant aggregations of slugs, areas where more slugs were found than would be expected if the slugs were randomly distributed across the field at $p<0.05$ significance level. Purple shows the areas of the field where the number of slugs observed was not significantly different to that expected if they were randomly distributed.

3.3.2.2. Taylor's Power law

The index of aggregation varied between years (Table 3.33), being highest in the year in which larger slug counts were recorded (1.67 in 2015-16) and lowest in the year with the fewest slugs (0.88 in 2016-17). According to Taylor's Power law if a species has a regular distribution the index of aggregation tends towards zero, an index of aggregation close to 1 suggests a random distribution, the higher the index of aggregation above 1 the more aggregated the species is, with an index of aggregation above 2 considered to show that the species is highly aggregated (Taylor, 1961). These results suggest that in the years when larger populations of surface active slugs were recorded the distribution is more highly aggregated than in years when numbers were lower (Table 3.33; Figure 3.1010).

Table 3.3. Index of aggregation (b) of Taylors Power Law calculated using slug counts taken during the three cropping seasons between 2015-16 and 2017-18, and for the combined data collected in all three seasons.

Year	Mean slug count	Index of aggregation (b)
2015-16	451.3	1.67
2016-17	55.5	0.88
2017-18	145.7	1.05
2015-18	170.0	1.10

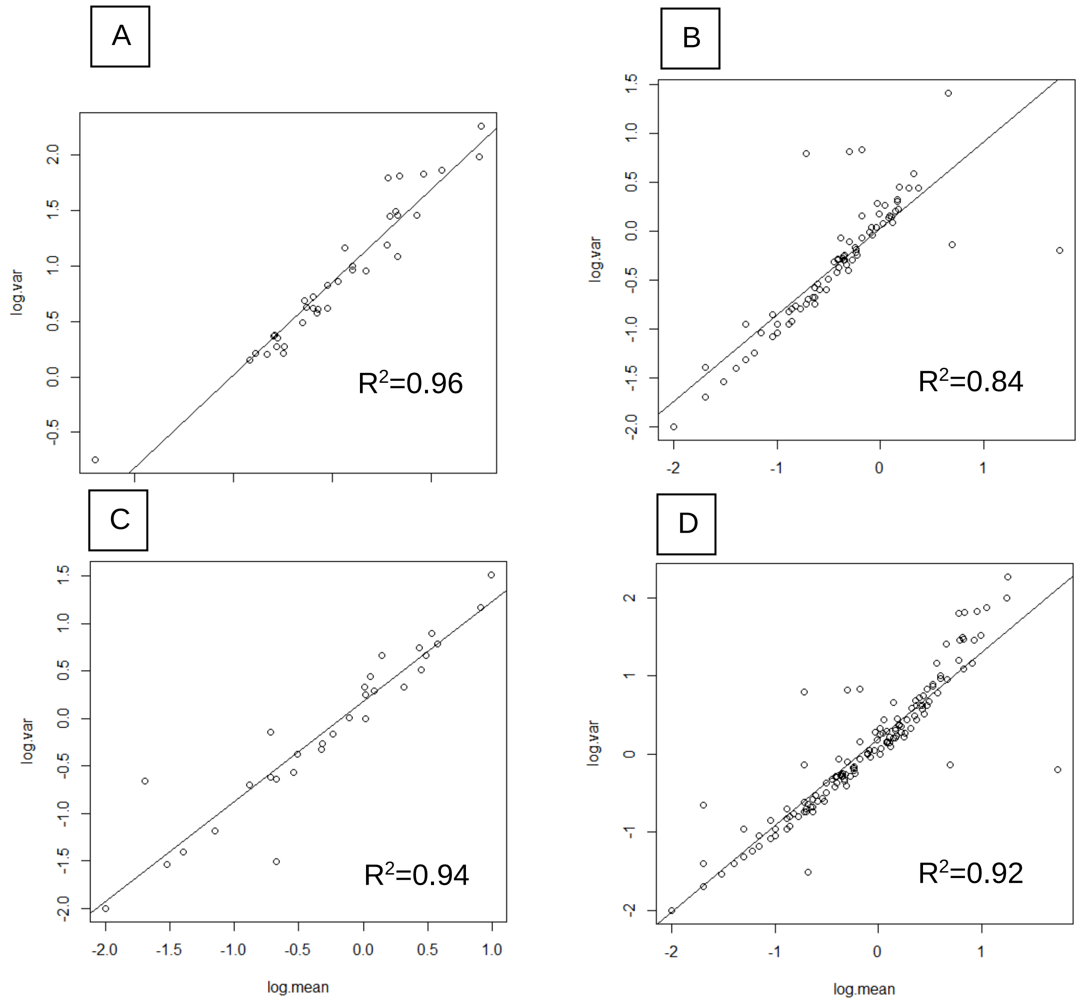


Figure 3.10. Calculation of the index of aggregation (b) of Taylors Power Law: Regression lines for the relationship between log S^2 against log mean, for slug counts taken in arable fields in 2015-16 (A), 2016-17 (B), 2017-18 (C) and the combined data for all three years, 2015-18 (D). Each point represents a single assessment at a field site.

3.3.3. Within season stability of slug patches

3.3.3.1. 2015-16 season

There was variation in the number of slugs that were active on the soil surface (reflected in refuge trap catches) between assessment visits and field sites (Table 3.44). Patches of higher slug numbers were located in all five fields during the 2015-16 season, the size and shape of these patches varied between fields but remained spatially stable within each field, with patches ranging from 300 to 7000 m^2. Throughout the 2015-16 growing season the locations of individual slug patches were highly correlated between assessments (Table 3.55). At Adeney (Corner) correlations as high as r = 0.38 (t=4.09, d.f.=98, p<0.001) were observed between assessments, at Adeney (Middle) the highest correlation was r = 0.65 (t=8.46, d.f.=98, p<0.001), Lynn (Badjics) r = 0.53 (t=6.20,d.f.=98, p<0.001), Lynn (Stoney Lawn) r = 0.85 (t=15.81, d.f.=98, p<0.001) and Uppington (1) r = 0.3 (t=3.12, d.f.=98, p=0.002). High correlations were not only observed between assessment sites in temporal proximity to each other but also across the season, for example, at Adeney (Middle) a correlation of r = 0.41 (t=4.46, d.f.=98, p<0.001) was observed between the assessment on 14/1/16 and 26/4/16, at Lynn (Badjics) a correlation of r = 0.4 (t=4.29, d.f.=98, p<0.001) was found between the assessment on 8/12/15 and 3/2/16 and at Lynn (Stoney Lawn) there was a correlation of r = 0.43 (t=4.68, d.f.=98, p<0.001) between assessments on 18/12/15 and 15/3/16. The correlations between trap counts demonstrates that the highest trap counts are reappearing in the same location, further work investigated whether traps in close proximity to each other displayed similar stability.

Figure 3.11 shows that when the surface activity of slugs at the Adeney (Corner) site was sufficiently high to identify areas of the field with an average count of more than 4 per trap, the location of these areas remained stable whenever they appeared (in this case they were apparent in four of the seven assessments). Within the stable area of higher slug numbers, the individual trap recording the highest count could vary between assessment dates. A similar pattern of stability was observed at the other field sites monitored in the 2015-16 season, with the area of the field with the highest number of slugs occurring in the same location in nine out of eleven assessments of Adeney (Middle) (Figure 3.12), six out of seven in Lynn (Badjics) (Figure 3.13), 11 out of 13 in Lynn (Stoney Lawn) (Figure 3.14), and of the two areas containing hotspots in Uppington (1), one occurred on six out of eight assessment dates and the other four out of eight (Figure 3.15).

Table 3.4. Maximum total slug count and maximum individual trap count in arable fields assessed during the 2015-16 field season (between November 2015 and May 2016). 100 refuge traps were set up in each field in a 10 by 10 grid at 10 metre intervals.

Field	Maximum total count	Maximum individual trap count
Adeney (Corner)	133	7
Adeney (Middle)	677	33
Lynn (Badjics)	400	14
Lynn (Stoney Lawn)	1796	143
Uppington (1)	673	23

Table 3.5. Pearson's Product Moment Correlation coefficient (r) between slug counts from 100 refuge traps in Adeney (Corner), Adeney (Middle), Lynn (Badjics), Lynn (Stoney Lawn) and Uppington (1) on assessment dates between November 2015 and May 2016. Refuge traps were set up in a 10 by 10 grid at 10 metre intervals. Significant correlations between trap counts on different assessment dates are highlighted in yellow.

Adeney (Middle)	08/12/2015	22/12/2015	05/01/2016	11/01/2016	14/01/2016	18/01/2016	25/01/2016	29/01/2016	09/02/2016	12/02/2016
08/12/2015										
22/12/2015	-0.02									
05/01/2016	-0.13	0.24								
11/01/2016	-0.21	0.25	0.27							
14/01/2016	0.01	0.13	0.23	0.58						
18/01/2016	-0.19	0.22	0.32	0.65	0.64					
25/01/2016	-0.06	0.24	0.28	0.49	0.60	0.65				
29/01/2016	0.05	0.32	0.21	0.29	0.35	0.30	0.52			
09/02/2016	-0.08	0.05	0.32	0.47	0.46	0.49	0.45	0.41		
12/02/2016	-0.15	0.14	0.37	0.19	0.31	0.26	0.29	0.19	0.35	
26/04/2016	0.11	0.16	0.23	0.32	0.41	0.34	0.37	0.25	0.32	0.29

Adeney (Corner)	30/11/2015	14/12/2015	05/01/2016	15/01/2016	29/01/2016	16/02/2016
30/11/2015						
14/12/2015	0.24					
05/01/2016	0.01	0.15				
15/01/2016	-0.09	0.05	0.27			
29/01/2016	-0.01	0.15	0.22	0.06		
16/02/2016	0.01	0.06	0.08	0.38	0.07	
26/04/2016	0.14	0.08	-0.22	-0.01	0.00	0.16

Lynn (Badjics)	08/12/2015	18/12/2015	06/01/2016	20/01/2016	03/02/2016	18/02/2016
08/12/2015						
18/12/2015	0.37					
06/01/2016	0.24	0.13				
20/01/2016	0.26	0.26	0.16			
03/02/2016	0.40	0.33	0.08	0.16		
18/02/2016	0.36	0.36	0.10	0.42	0.53	
25/05/2016	-0.01	-0.17	-0.03	-0.08	0.12	0.13

Lynn (Stoney Lawn)	07/12/2015	18/12/2015	22/12/2016	06/01/2016	11/01/2016	14/01/2016	21/01/2016	26/01/2016	02/02/2016	18/02/2016	15/03/2016	02/05/2016
07/12/2015												
18/12/2015	0.29											
22/12/2016	0.24	0.45										
06/01/2016	0.23	0.64	0.51									
11/01/2016	0.19	0.53	0.48	0.62								
14/01/2016	0.12	0.52	0.59	0.64	0.83							
21/01/2016	0.11	0.52	0.54	0.58	0.80	0.85						
26/01/2016	0.23	0.59	0.65	0.57	0.65	0.66	0.73					
02/02/2016	-0.02	0.34	0.57	0.43	0.47	0.61	0.53	0.59				
18/02/2016	0.04	0.42	0.58	0.54	0.73	0.79	0.73	0.65	0.68			
15/03/2016	0.02	0.43	0.55	0.50	0.66	0.67	0.72	0.64	0.52	0.71		
02/05/2016	0.15	0.26	0.27	0.29	0.27	0.34	0.29	0.37	0.30	0.21	0.25	
24/05/2016	0.00	0.14	0.22	0.14	0.14	0.17	0.22	0.32	0.32	0.17	0.33	0.41

Uppington (1)	07/12/2015	17/12/2015	04/01/2016	19/01/2016	01/02/2016	16/02/2016	29/04/2016
07/12/2015							
17/12/2015	0.28						
04/01/2016	0.00	0.07					
19/01/2016	0.00	-0.09	0.11				
01/02/2016	0.18	-0.03	-0.13	-0.01			
16/02/2016	-0.03	-0.03	0.10	0.19	0.03		
29/04/2016	0.11	-0.03	-0.07	0.12	0.03	0.00	
23/05/2016	0.05	0.16	0.30	0.27	-0.17	0.16	0.02

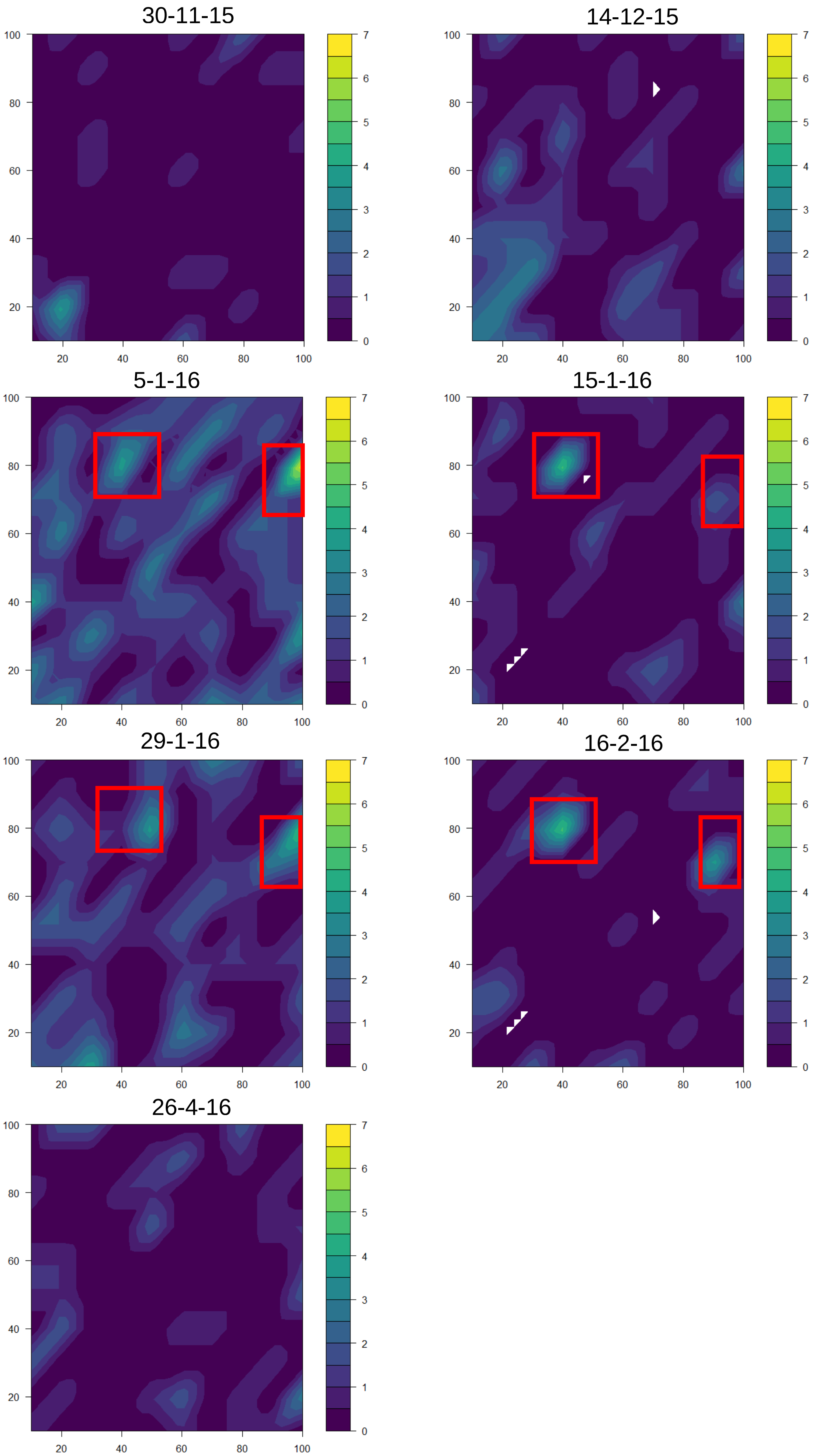
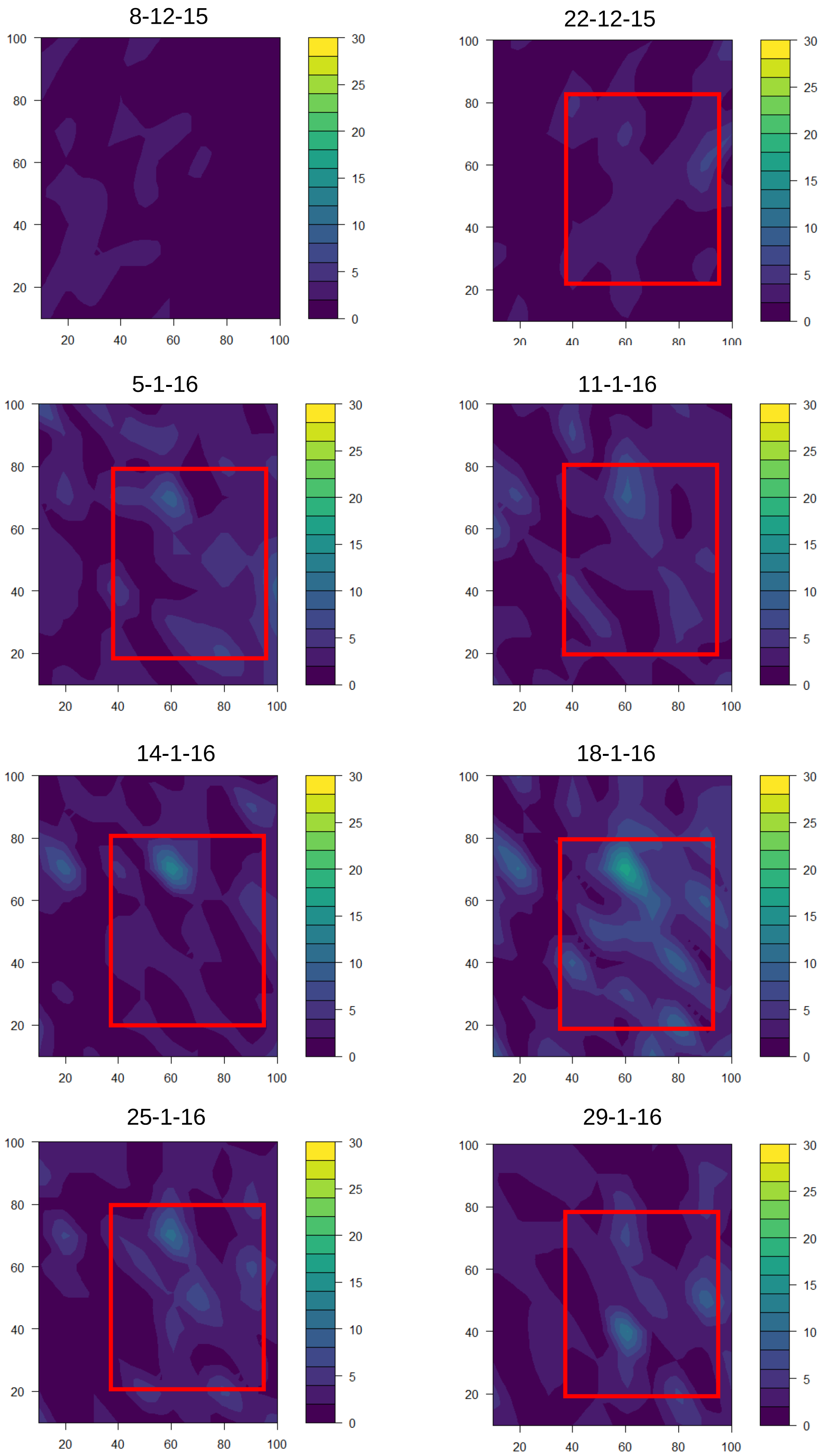


Figure 3.11. Heat maps showing slug distribution at Adeney (Corner) from assessments carried out between November 2015 and April 2016. The numbers along the x and y axis show dimensions of the sampling grid in metres. 100 refuge traps were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the number of slugs, with the numbers in between traps calculated by polynomial interpolation. The areas highlighted in red shows the location of the traps with the highest slug counts.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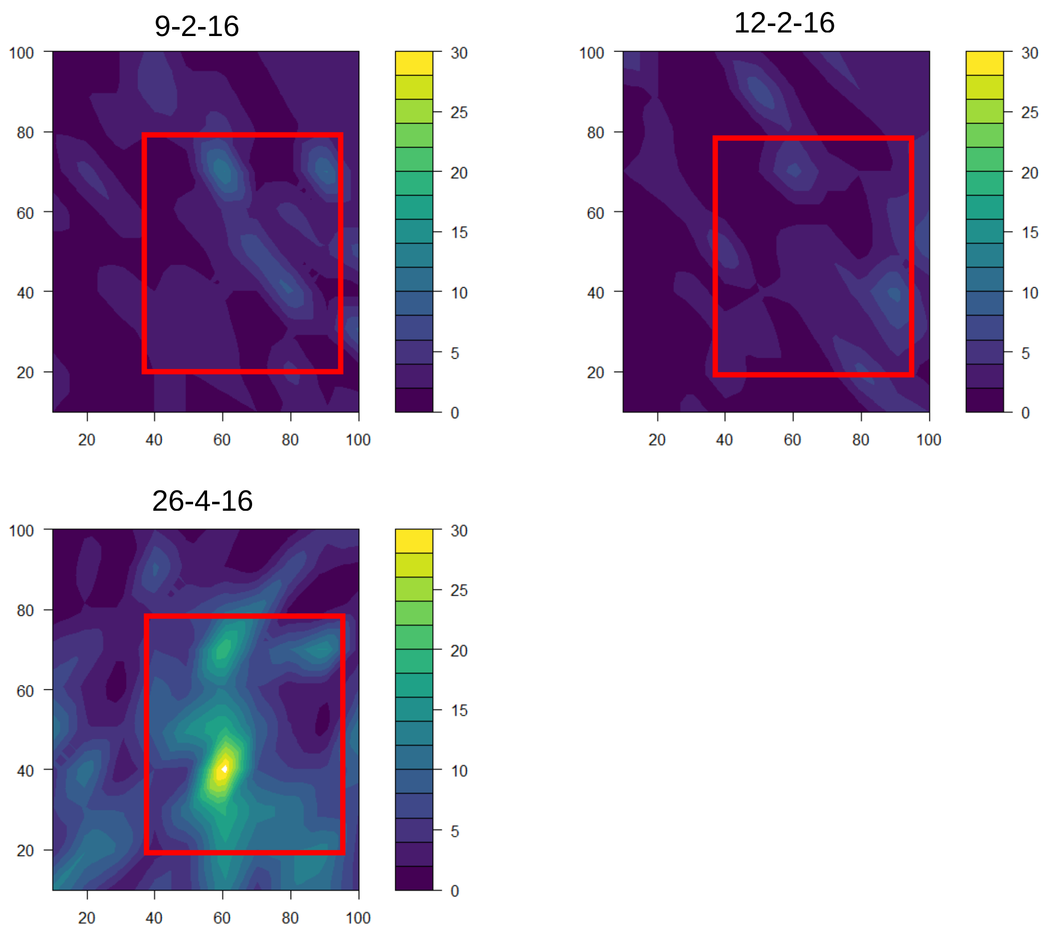


Figure 3.12. Heat maps showing slug distribution at Adeney (Middle) from assessments carried out between December 2015 and April 2016. The numbers along the x and y axis show dimensions of the sampling grid in metres. 100 refuge traps were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the number of slugs, with the numbers in between traps calculated by polynomial interpolation. The areas highlighted in red shows the location of the traps with the highest slug counts.

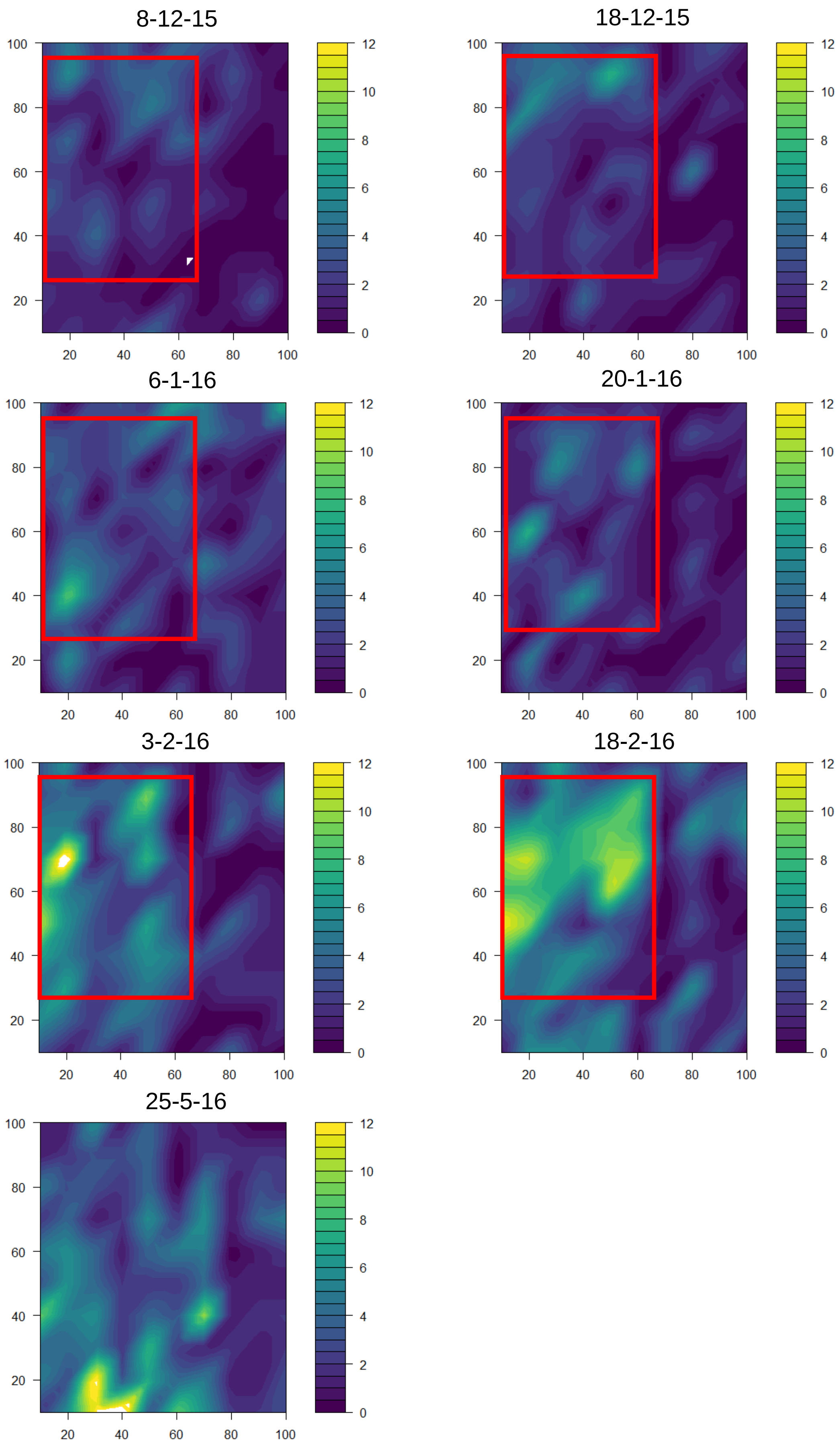
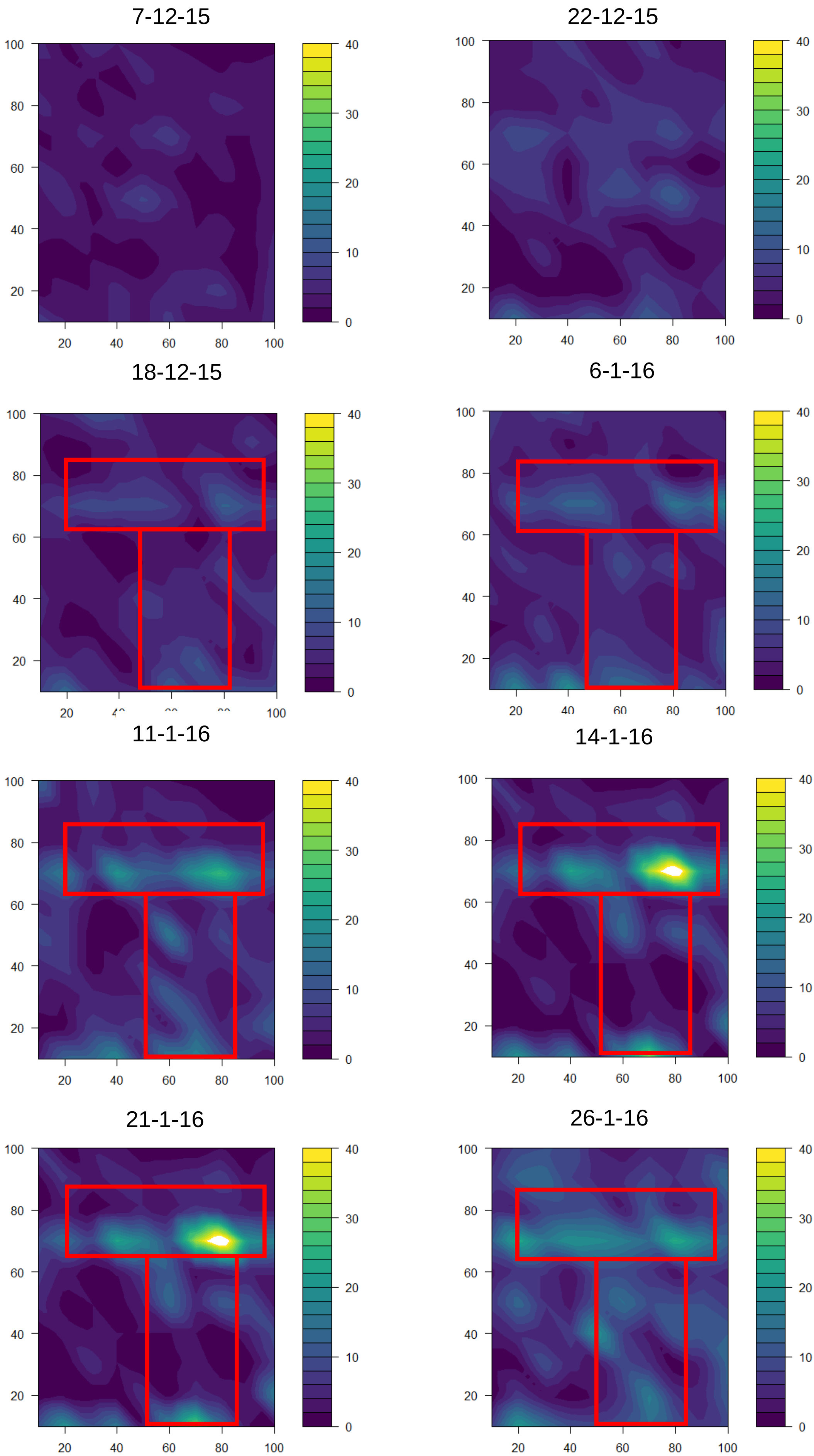


Figure 3.13. Heat maps showing slug distribution at Lynn (Badjics) from assessments carried out between December 2015 and May 2016. The numbers along the x and y axis show dimensions of the sampling grid in metres. 100 refuge traps were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the number of slugs, with the numbers in between traps calculated by polynomial interpolation. The areas highlighted in red shows the location of the traps with the highest slug counts.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21-1-16
26-1-16

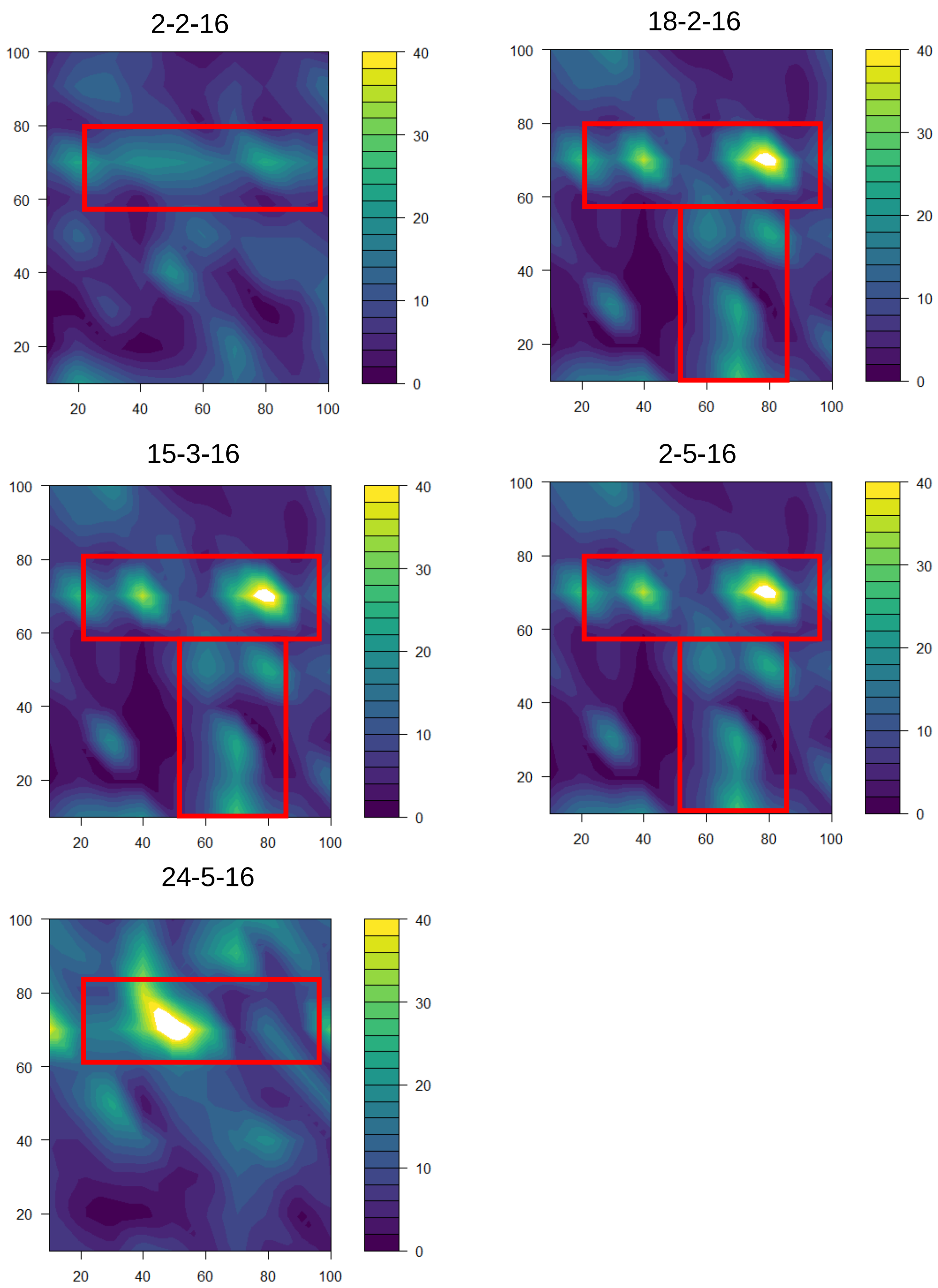


Figure 3.14. Heat maps showing slug distribution at Lynn (Stoney Lawn) from assessments carried out between December 2015 and May 2016. The numbers along the x and y axis show dimensions of the sampling grid in metres. 100 refuge traps were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the number of slugs, with the numbers in between traps calculated by polynomial interpolation. The areas highlighted in red shows the location of the traps with the highest slug counts.

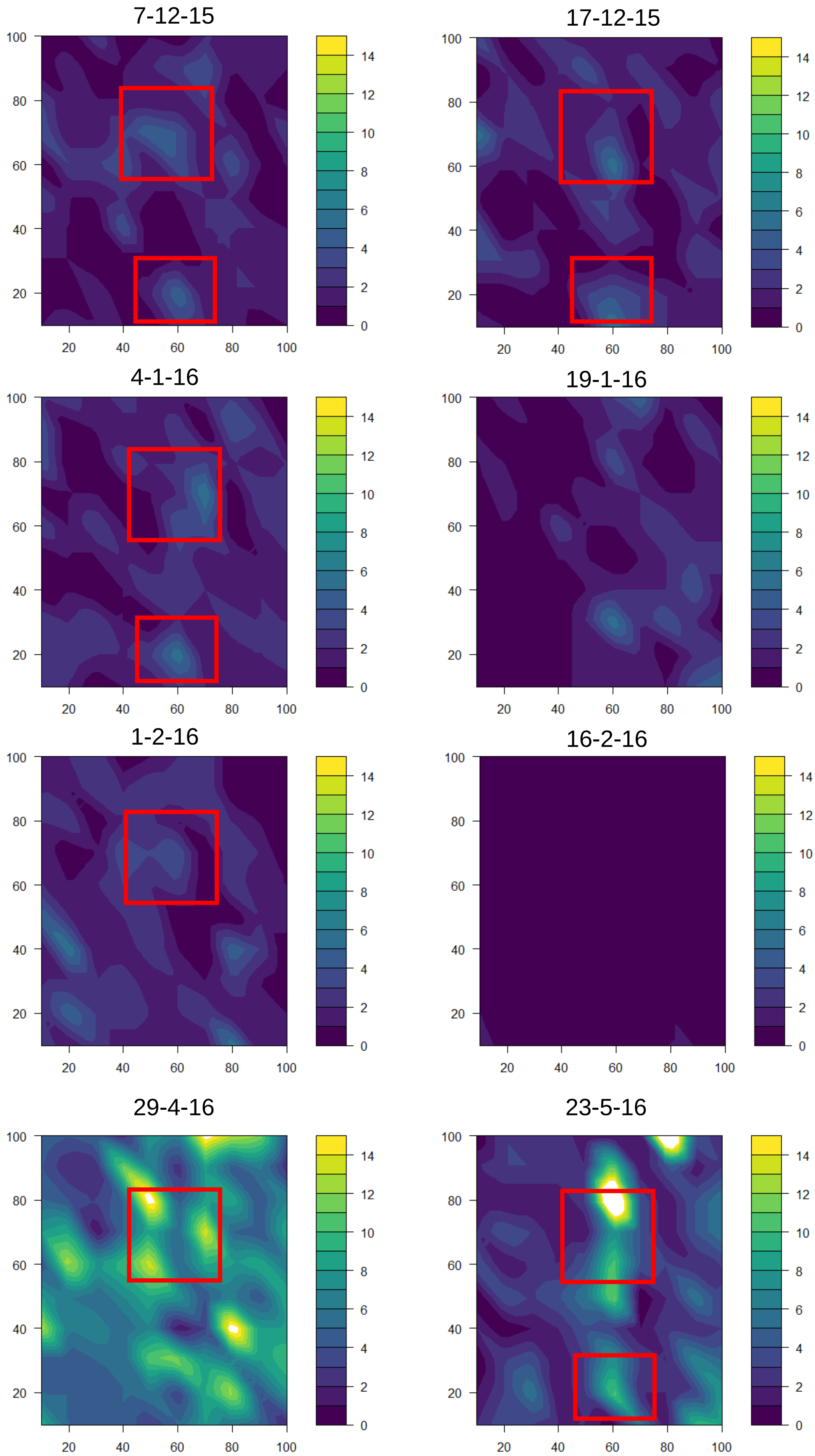


Figure 3.15. Heat maps showing slug distribution at Uppington (1) from assessments carried out between December 2015 and May 2016. The numbers along the x and y axis show dimensions of the sampling grid in metres. 100 refuge traps were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the number of slugs, with the numbers in between traps calculated by polynomial interpolation. The areas highlighted in red shows the location of the traps with the highest slug counts.

3.3.3.2. 2016-17 season

The total number of slugs observed in 2016-17 was significantly lower ($F= 41.74$, d.f.= 2, 157, $p<0.001$) than the 2015-16 season (mean of 55.5 ±7.0 slugs recorded per assessment date compared with 451.3 ±63.8 in 2015-16). The average number of slugs per trap was below the AHDB threshold level (average 4 slugs per trap in a standing crop, (AHDB, 2016)) on all assessment dates at all field sites. In fields where slug numbers were lowest there was little variation between the maximum and minimum slug catches in individual refuge traps (Table 3.66). The generally lower catches in individual refuge traps also resulted in no distinct patches being detectable in some fields, as illustrated by the heat map for Adeney (Middle) (Figure 3.16). Despite the low slug populations in the two fields with the highest maximum total counts (Uppington (1) and South Kyme (1)) and in one field with a low population (Wigan) (Table 3.66), some correlations between trap counts on different assessment dates were found. The highest correlations were in South Kyme (1), $r = 0.33$ ($t=3.41$, d.f.=98, $p<0.001$) between assessments on 17/2/17 and 9/3/17, Uppington (1), $r = 0.38$ ($t=4.11$, d.f.=98, $p<0.001$) between assessments on 13/9/16 and 28/2/17 and Wigan, $r = 0.53$ ($t=6.23$, d.f.=98, $p<0.001$) between assessments on 12/4/17 and 10/5/17 (Table 3.77). In these field, similar patterns of stability to those detected in the 2015-16 field season were observed, in Uppington (1) on all seven assessments (Figure 3.17). Slightly lower populations resulted in clusters of patches of higher slug areas being more difficult to identify, but the areas they formed were still visible in South Kyme (1) on five out of nine assessments (Figure 3.18) and in Wigan on seven out of ten assessments (Figure 3.199).

Table 3.6. Maximum total slug count and maximum individual trap count in each field assessed in the 2016-17 field season (between August 2016 and May 2017). Refuge traps were set up in each field in a 10 by 10 grid at 10 metre intervals.

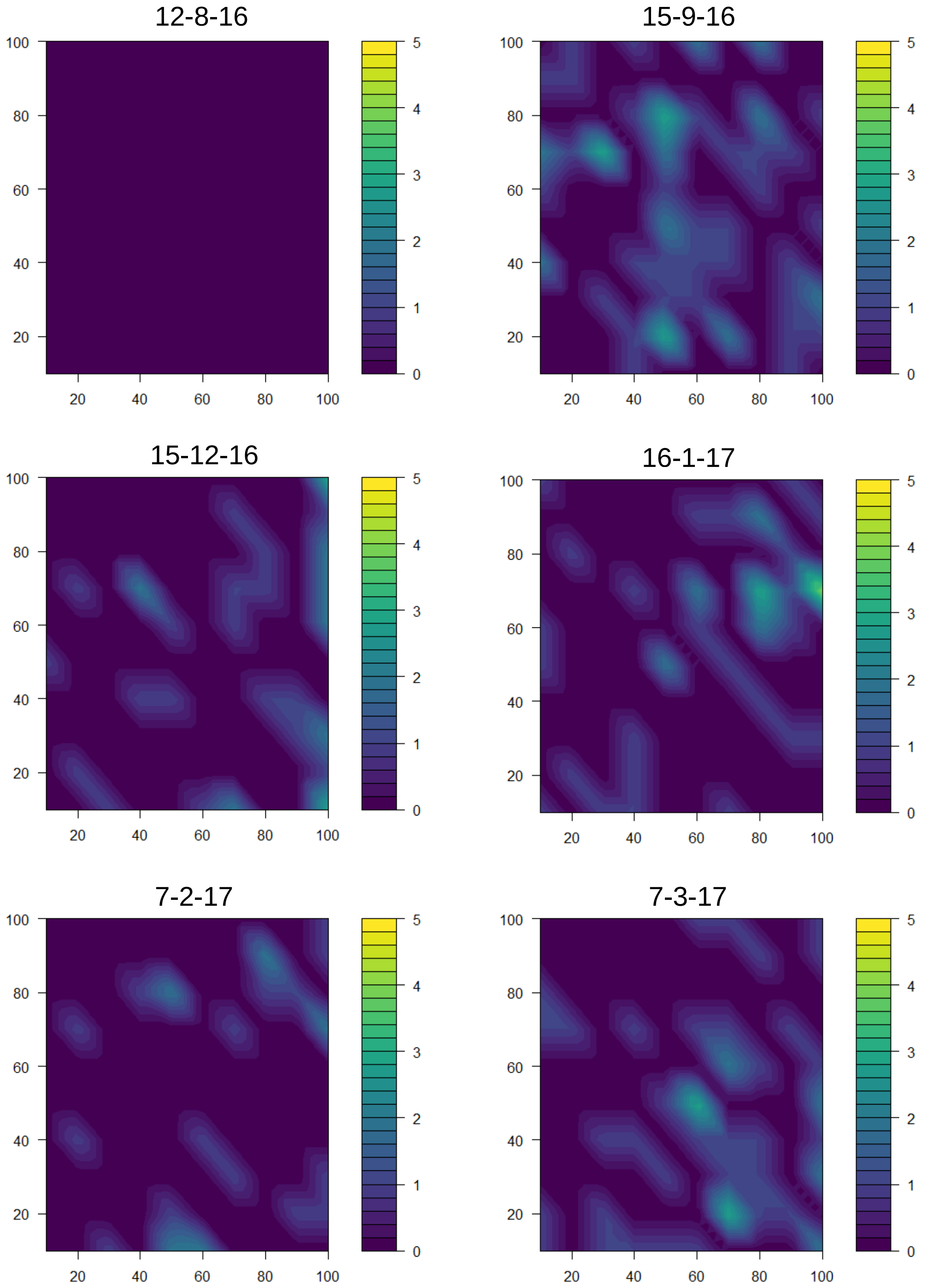
Field	Maximum total count	Minimum individual trap count	Maximum individual trap count
Adeney (Middle)	58	0	4
Lynn (Badjics)	44	0	4
Bridgnorth	52	0	3
Dogdyke	20	0	3
Flawborough	45	0	3
Hoby	132	0	6
Oadby	107	0	6
South Kyme (1)	151	0	6
South Kyme (2)	57	0	5
Lynn (Stoney Lawn)	67	0	10
Uppington (1)	225	0	10
Wigan	90	0	24

Table 3.7. Pearsons Correlation coefficient (r) between slug counts from 100 refuge traps in South Kyme (1), Uppington (1) and Wigan on assessment dates between August 2016 and June 2017. Refuge traps were set up in a 10 by 10 grid at 10 metre intervals. Significant correlations between trap counts on different assessment dates are highlighted in yellow.

South Kyme (1)	21/08/2016	25/11/2016	22/12/2016	24/01/2017	17/02/2017	09/03/2017	31/03/2017	24/04/2017
21/08/2016								
25/11/2016								
22/12/2016		-0.01						
24/01/2017		0.14	-0.01					
17/02/2017		-0.10	0.07	0.11				
09/03/2017		0.15	-0.04	0.05	0.33			
31/03/2017		0.18	0.05	0.23	0.13	0.24		
24/04/2017		0.00	-0.15	0.13	0.05	0.05	0.27	
24/05/2017		0.13	-0.01	0.12	-0.07	0.15	0.02	-0.06

Uppington (1)	13/09/2016	18/10/2016	23/11/2016	20/12/2016	17/01/2017	10/02/2017
13/09/2016						
18/10/2016	0.07					
23/11/2016	-0.13	0.11				
20/12/2016	0.11	0.30	0.16			
17/01/2017	0.07	0.19	0.21	0.06		
10/02/2017	0.24	0.14	0.00	0.19	0.36	
28/02/2017	0.38	0.10	0.03	0.34	0.27	0.37

Wigan	20/10/2016	21/11/2016	18/01/2017	08/02/2017	02/03/2017	22/03/2017	12/04/2017	10/05/2017	08/06/2017
20/10/2016									
21/11/2016	0.10								
18/01/2017	0.05	-0.01							
08/02/2017	-0.01	-0.07	0.29						
02/03/2017	0.19	0.13	0.26	0.34					
22/03/2017	-0.11	0.05	0.21	0.12	0.07				
12/04/2017	0.00	0.05	0.15	0.17	0.07	0.40			
10/05/2017	-0.02	0.13	0.12	0.25	0.18	0.36	0.53		
08/06/2017	0.18	0.02	-0.09	-0.08	-0.03	-0.10	-0.01	-0.10	


12-8-16
15-9-16
15-12-16
16-1-17
7-2-17
7-3-17

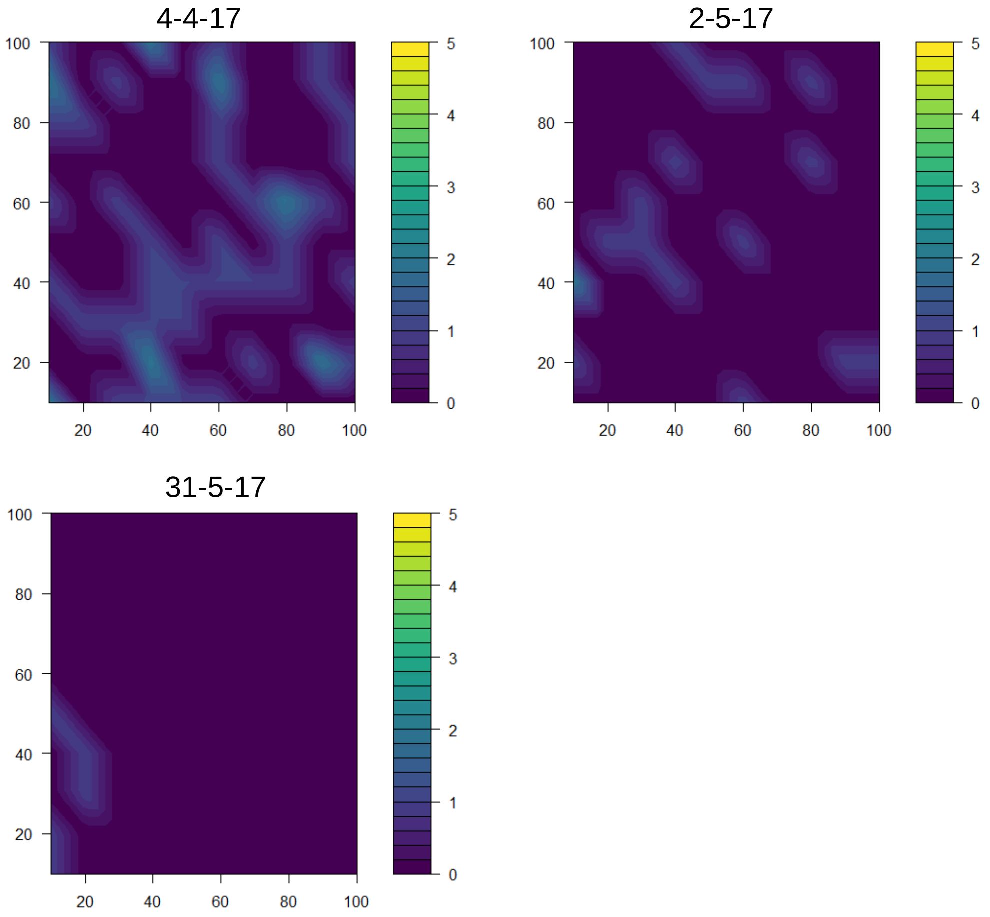


Figure 3.16. Heat maps showing slug distribution at Adeney (Middle) from assessments carried out between August 2016 and May 2017. The numbers along the x and y axis show dimensions of the sampling grid in metres. 100 refuge traps were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the number of slugs, with the numbers in between traps calculated by polynomial interpolation.

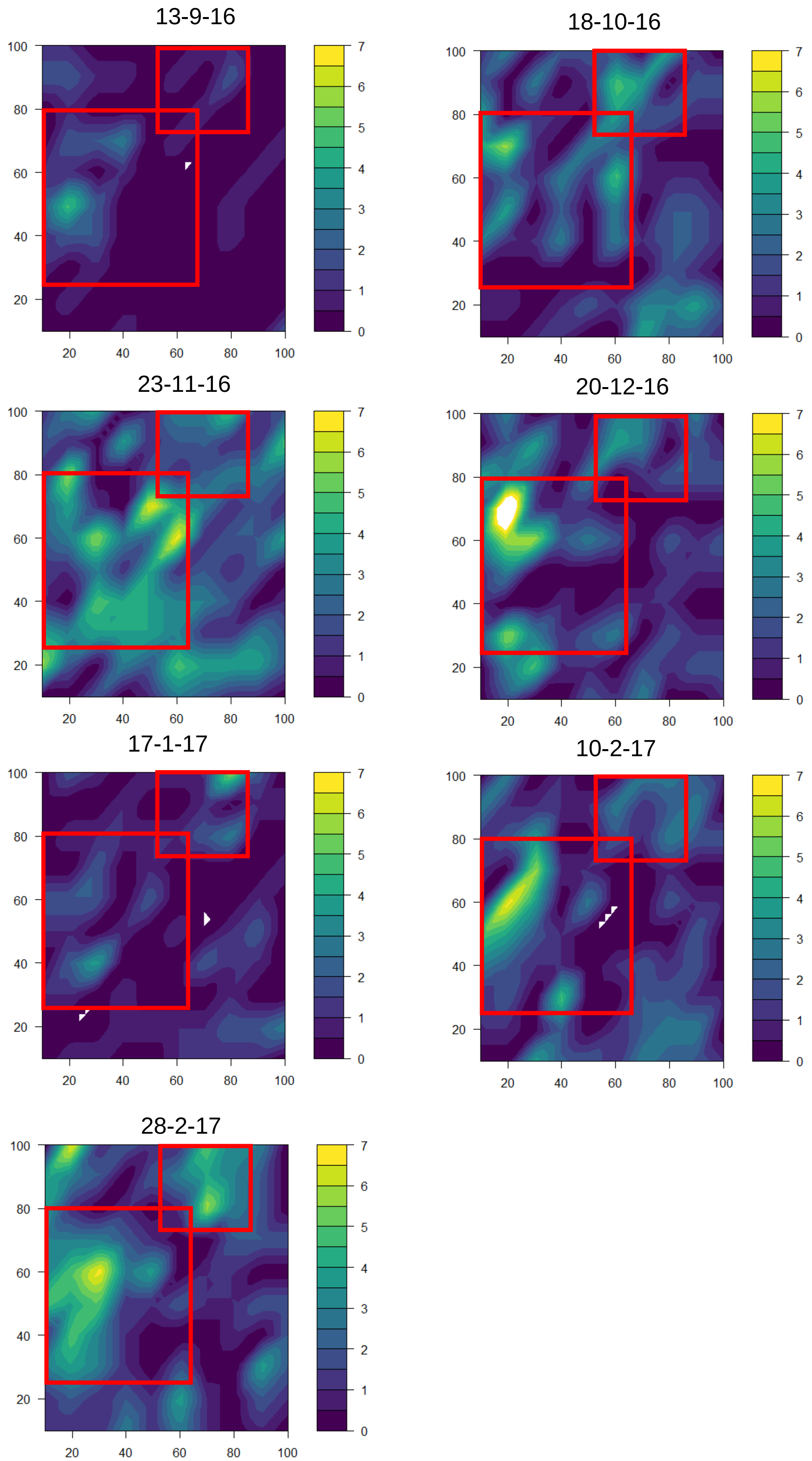
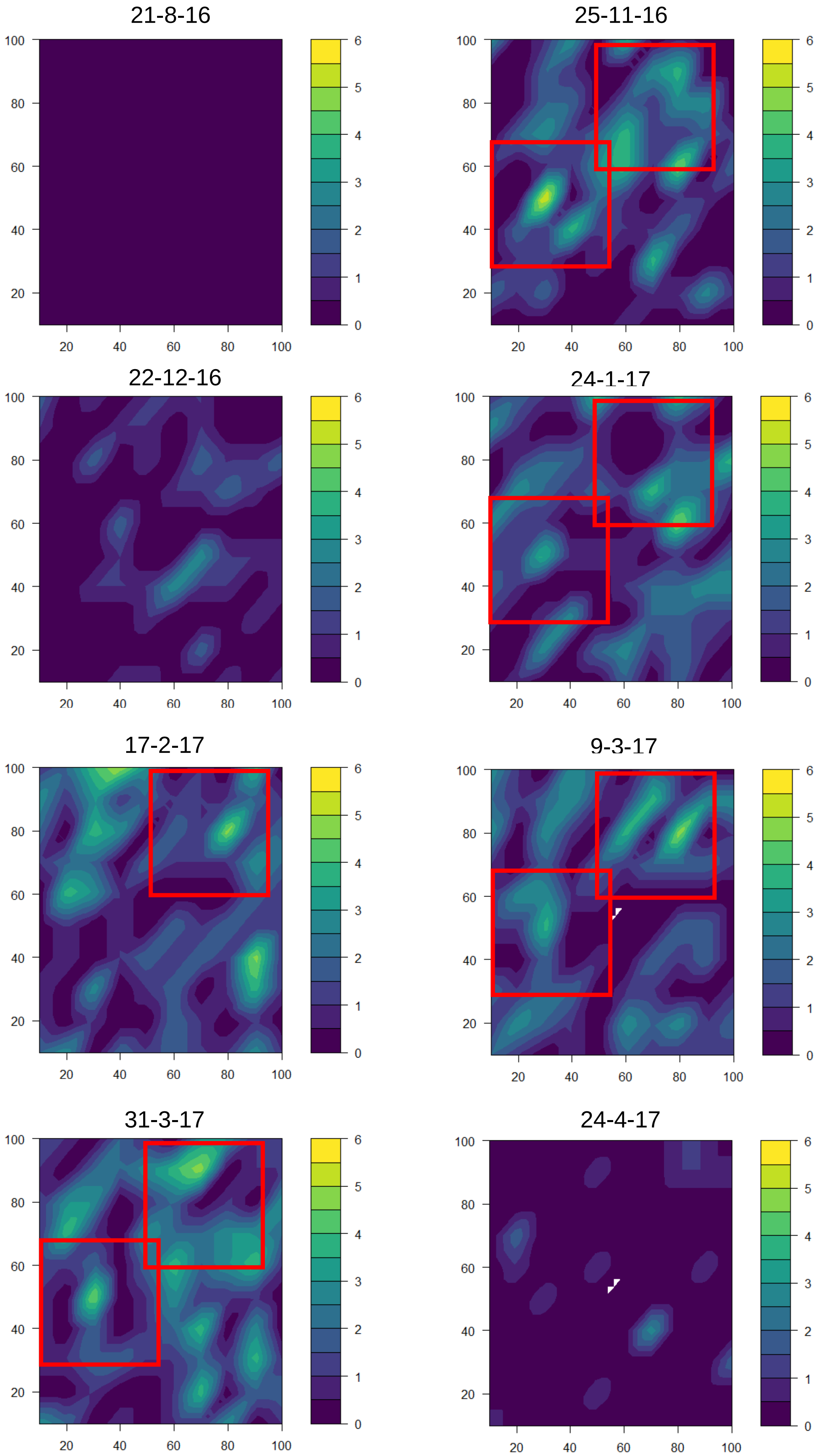


Figure 3.17. Heat maps showing slug distribution at Uppington (1) from assessments carried out between September 2016 and February 2017. The numbers along the x and y axis show dimensions of the sampling grid in metres. 100 refuge traps were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the number of slugs, with the numbers in between traps calculated by polynomial interpolation. The areas highlighted in red shows the location of the traps with the highest slug counts.


21-8-16
25-11-16
22-12-16
24-1-17
17-2-17
9-3-17
31-3-17
24-4-17

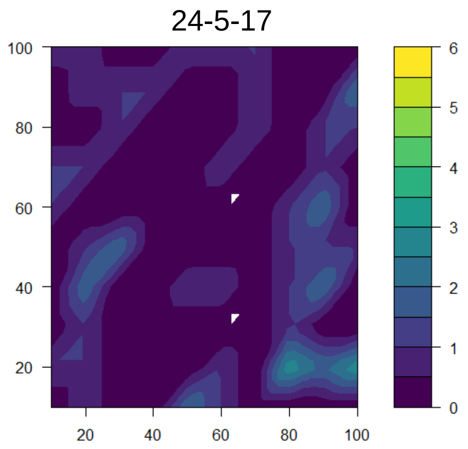
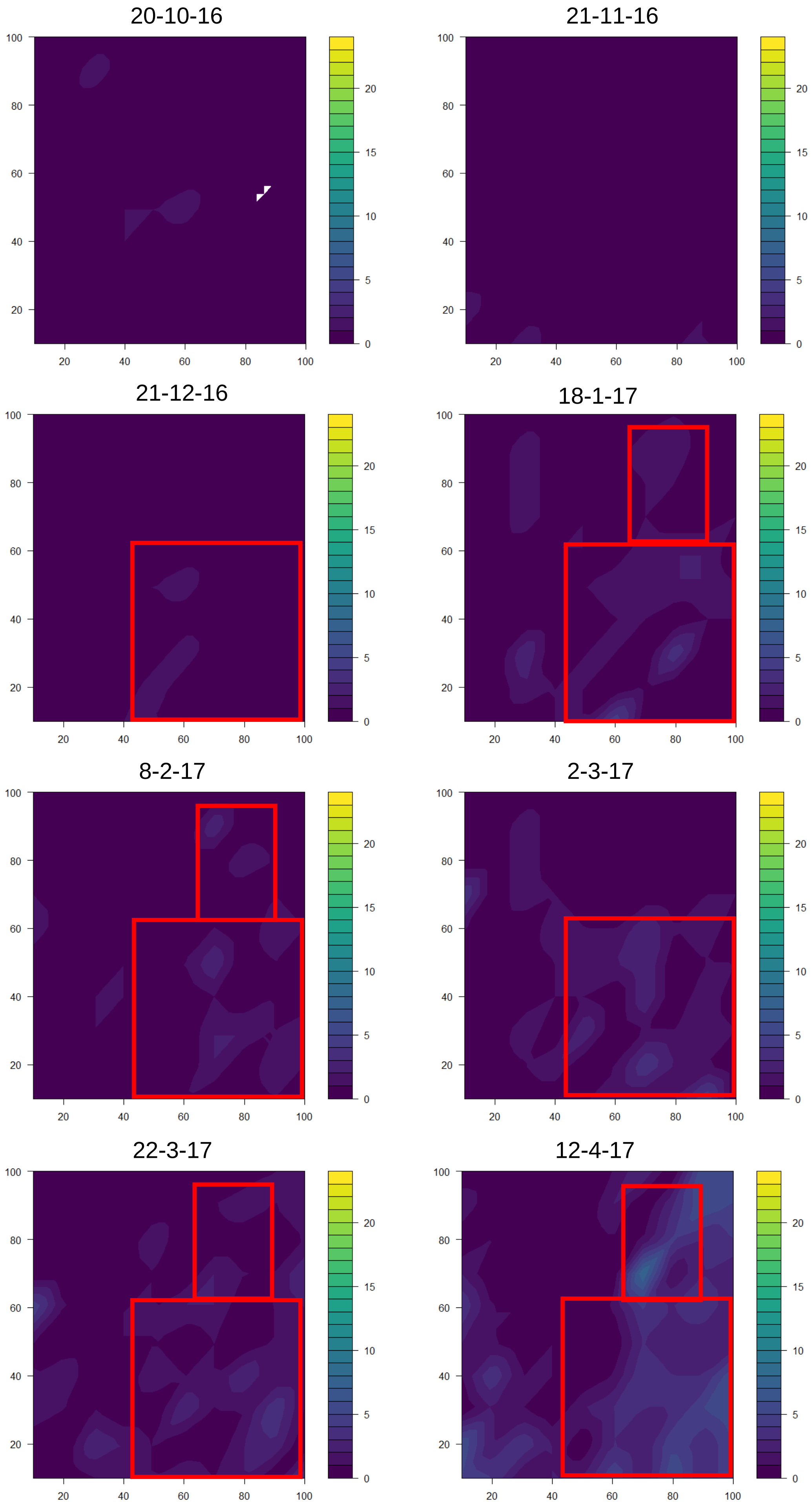


Figure 3.18. Heat maps showing slug distribution at South Kyme (1) from assessments carried out between August 2016 and May 2017. The numbers along the x and y axis show dimensions of the sampling grid in metres. 100 refuge traps were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the number of slugs, with the numbers in between traps calculated by polynomial interpolation. The areas highlighted in red shows the location of the traps with the highest slug counts.


20-10-16
21-11-16
21-12-16
18-1-17
8-2-17
2-3-17
22-3-17
12-4-17
100
80
60
40
20
20
40
60
80
100
0
5
10
15
20

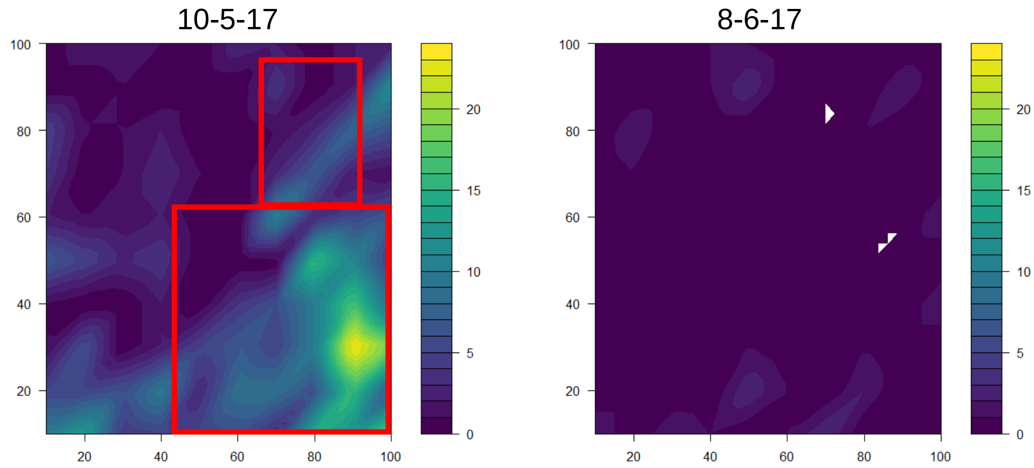


Figure 3.19. Heat maps showing slug distribution at Wigan from assessments carried out between October 2016 and June 2017. The numbers along the x and y axis show dimensions of the sampling grid in metres. 100 refuge traps were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the number of slugs, with the numbers in between traps calculated by polynomial interpolation. The areas highlighted in red shows the location of the traps with the highest slug counts.

3.3.3.4. 2017-18 season

The mean number of slugs observed at each assessment in 2017-18 was significantly higher than in 2016-17 and lower than in 2015-16 (mean of 145.7 ±40.2 slugs at each assessment compared to 55.5 ±7.0 in 2016-17 and 451.3 ±63.8 in 2015-16) ($F= 41.74$, d.f.= 2, 157, $p<0.001$). The variation between fields was high (Table 3.8), the mean total count, for each assessment visit, at Uppington (2) was 457 slugs compared with 26 at Oadby. At four of the five field sites where the slug counts were low (mean number of slugs per trap <2.7, in each case, lower than the AHDB 4/trap action threshold) no stable patches were detectable (Adeney (Middle), Figure 3.2020; Belchford, Figure 3.2121; Oadby, Figure 3.2222 and Wigan, Figure 3.2323). In Uppington (2), where the highest mean number of slugs was detected higher density patches were present and occurred in the same areas of the field on all seven assessment dates (Figure 3.24). The correlations between trap counts on different assessments dates were similar to the 2016-17 field season, the highest correlation was in Uppington (2) where $r = 0.47$ ($t=5.33$, d.f.=98, $p<0.001$) between assessments on 18/4/18 and 26/4/18 (Table 3.9).

Table 3.8. Maximum total slug count and maximum individual trap count in each field assessed in the 2017-18 field season (between September 2017 and June 2018). Refuge traps were set up in each field in a 10 by 10 grid at 10 metre intervals.

Field	Maximum total count	Maximum individual trap count
Adeney (Middle)	120	6
Belchford	267	17
Oadby	47	3
Uppington (2)	990	31
Wigan	205	8

Table 3.9. Pearson's Product Moment Correlation coefficient (r) between slug counts from 100 refuge traps in Adeney (Middle), Belchford, Uppington (2) and Wigan on assessment dates between September 2017 and June 2018. Refuge traps were set up in a 10 by 10 grid at 10 metre intervals. Significant correlations between trap counts on different assessment dates are highlighted in yellow.

Adeney (Middle)		28/09/2017	20/10/2017	20/12/2017	26/01/2018	08/03/2018	21/03/2018	15/05/2018
28/09/2017								
20/10/2017		-0.06						
20/12/2017		-0.05	0.02					
26/01/2018		-0.06	0.08	0.20				
08/03/2018		-0.05	0.06	0.03	0.05			
21/03/2018		0.03	0.18	0.32	0.33	-0.02		
15/05/2018		-0.03	-0.08	0.04	0.12	-0.05	-0.04	
30/05/2018		-0.06	-0.02	-0.15	-0.03	-0.05	-0.04	-0.06

Belchford		01/12/2017	05/01/2018	07/02/2018	14/03/2018	20/04/2018
01/12/2017						
05/01/2018		0.04				
07/02/2018		0.06	0.23			
14/03/2018		-0.07	0.11	0.18		
20/04/2018		-0.11	-0.04	-0.15	0.12	
01/06/2018		n/a	n/a	n/a	n/a	n/a

Uppington (2)	03/11/2017	20/12/2017	26/01/2018	09/03/2018	18/04/2018	26/04/2018
03/11/2017						
20/12/2017	-0.04					
26/01/2018	0.11	0.22				
09/03/2018	-0.22	0.16	0.15			
18/04/2018	0.06	0.05	0.21	0.21		
26/04/2018	0.06	-0.10	0.09	-0.04	0.47	
30/05/2018	-0.04	0.01	-0.08	0.04	0.09	0.29

Wigan	07/12/2017	12/01/2018	16/02/2018	20/03/2018
07/12/2017				
12/01/2018	-0.04			
16/02/2018	0.18	0.14		
20/03/2018	0.08	0.07	0.15	
11/04/2018	0.15	0.08	0.21	-0.02

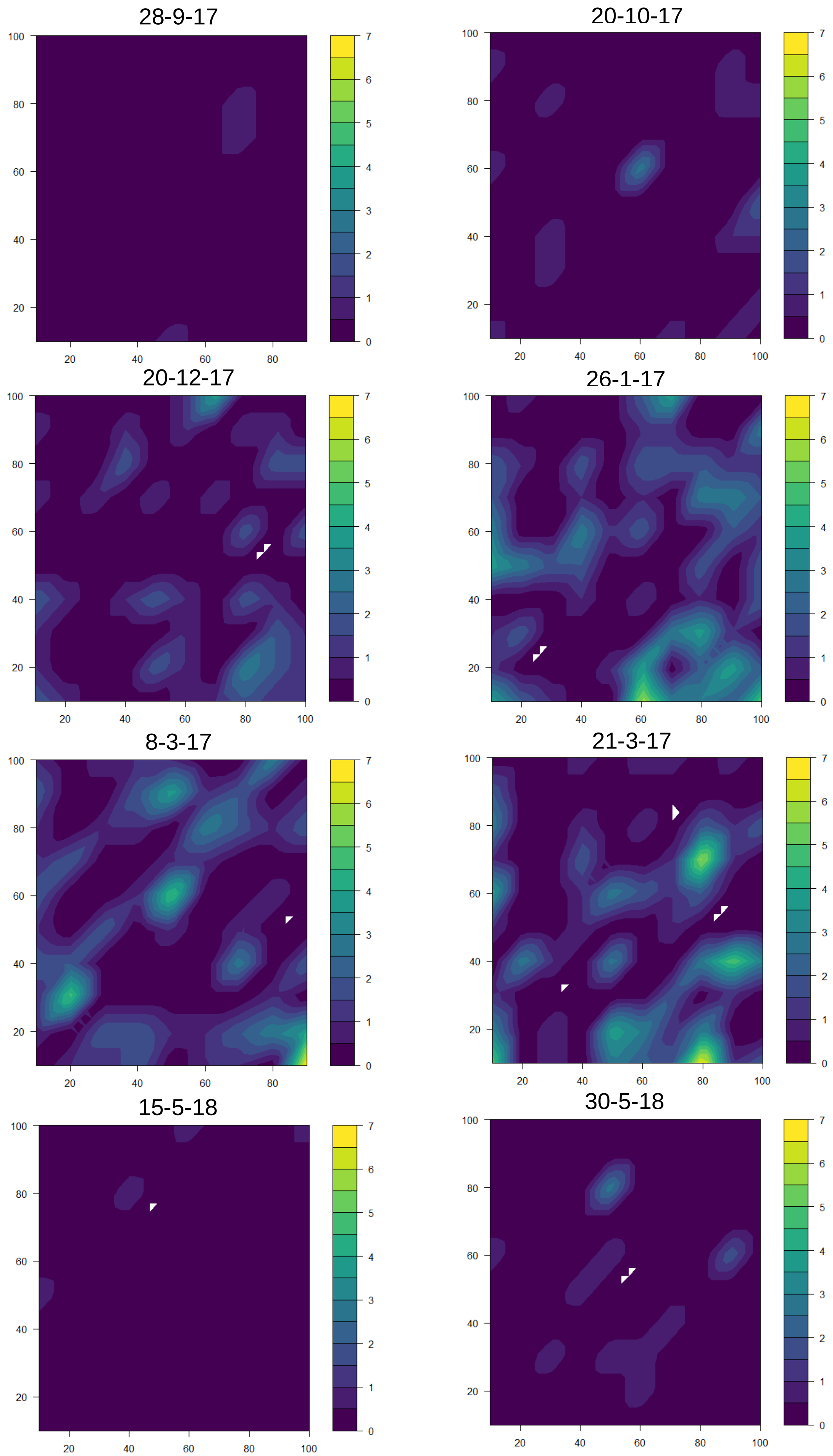


Figure 3.20. Heat maps showing slug distribution at Adeney (Middle) from assessments carried out between September 2017 and May 2018. The numbers along the x and y axis show dimensions of the sampling grid in metres. 100 refuge traps were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the number of slugs, with the numbers in between traps calculated by polynomial interpolation.

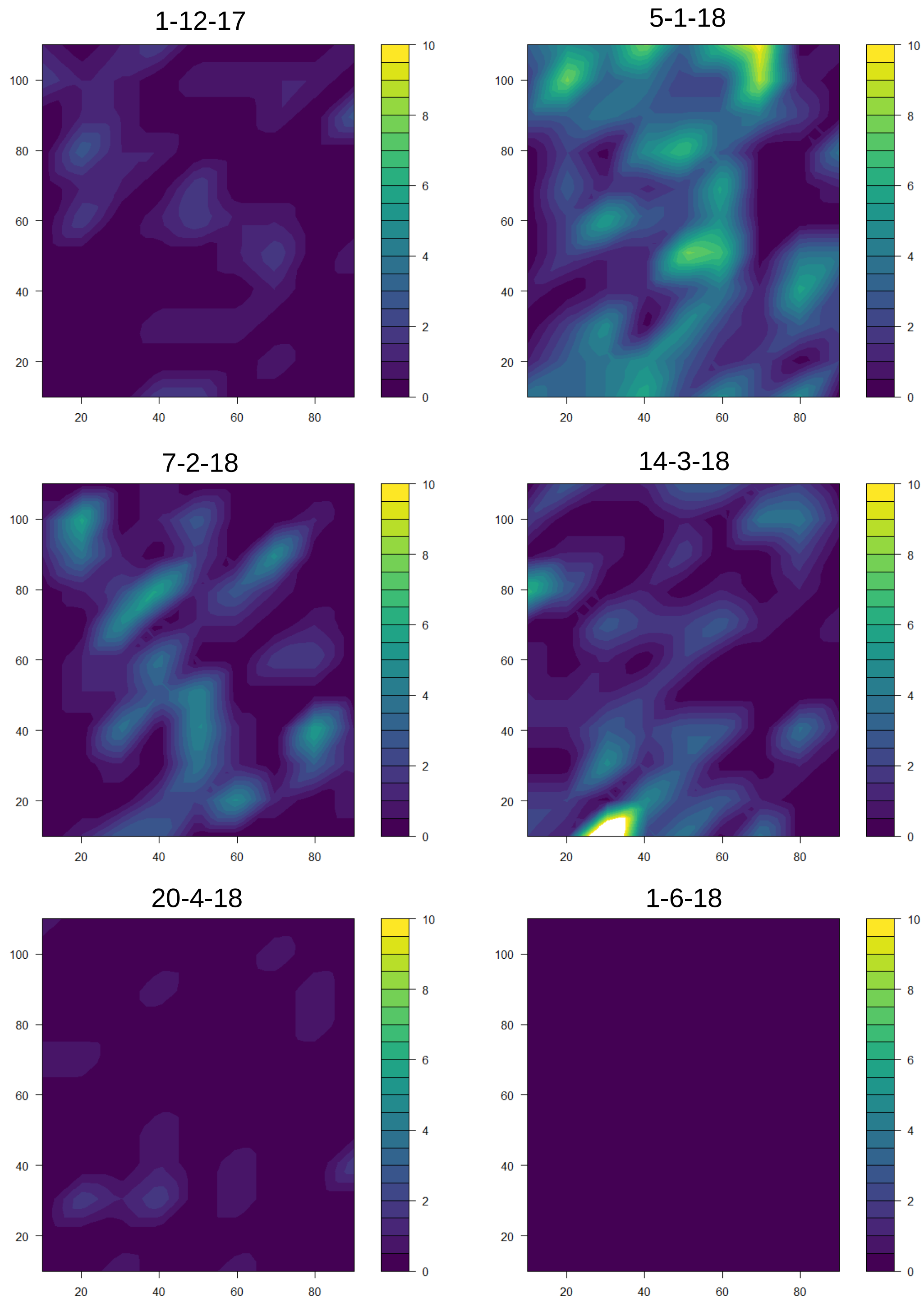


Figure 3.21. Heat maps showing slug distribution at Belchford from assessments carried out between December 2017 and June 2018. The numbers along the x and y axis show dimensions of the sampling grid in metres. 100 refuge traps were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the number of slugs, with the numbers in between traps calculated by polynomial interpolation.

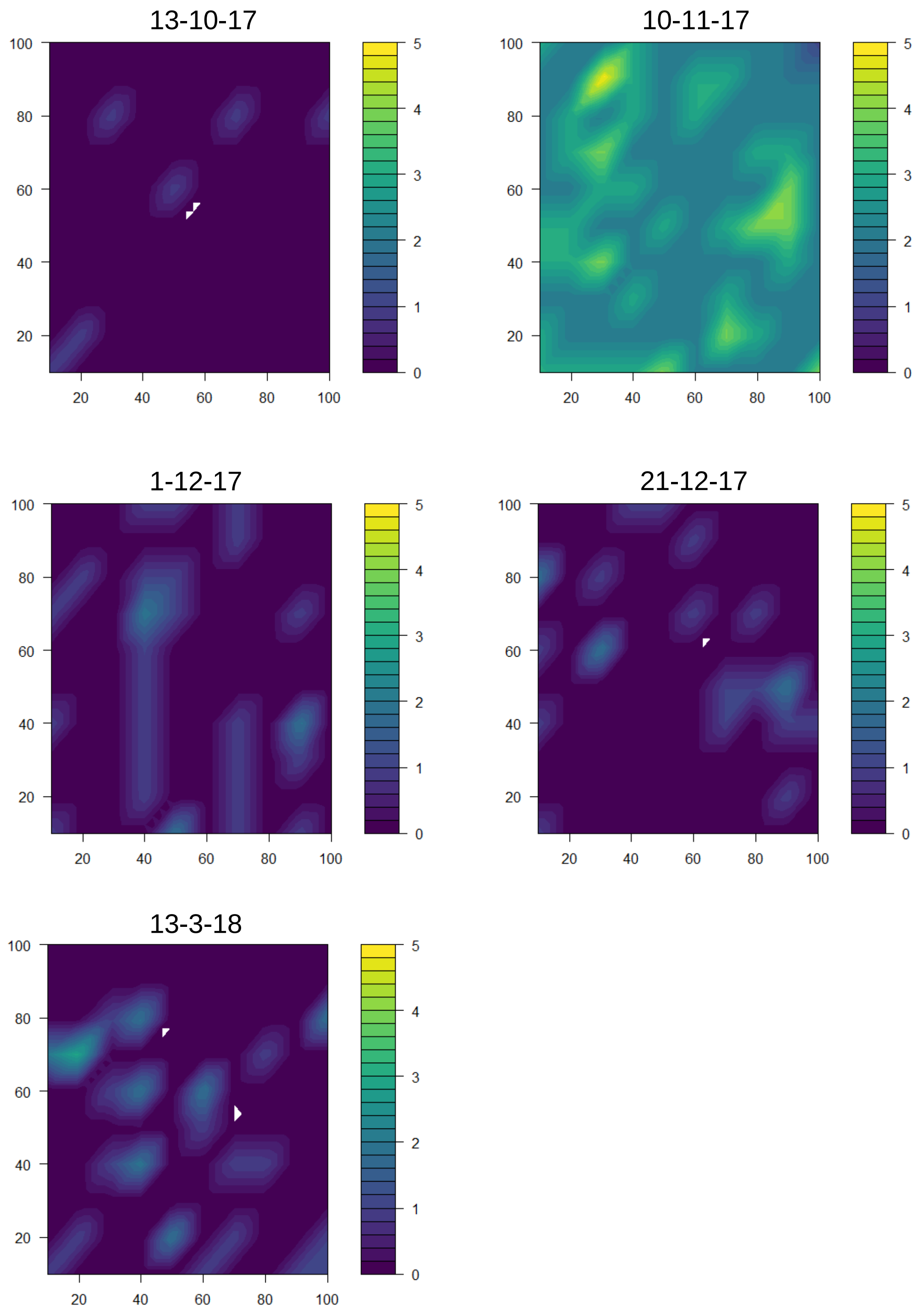


Figure 3.22. Heat maps showing slug distribution at Oadby from assessments carried out between October 2017 and March 2018. The numbers along the x and y axis show dimensions of the sampling grid in metres. 100 refuge traps were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the number of slugs, with the numbers in between traps calculated by polynomial interpolation.

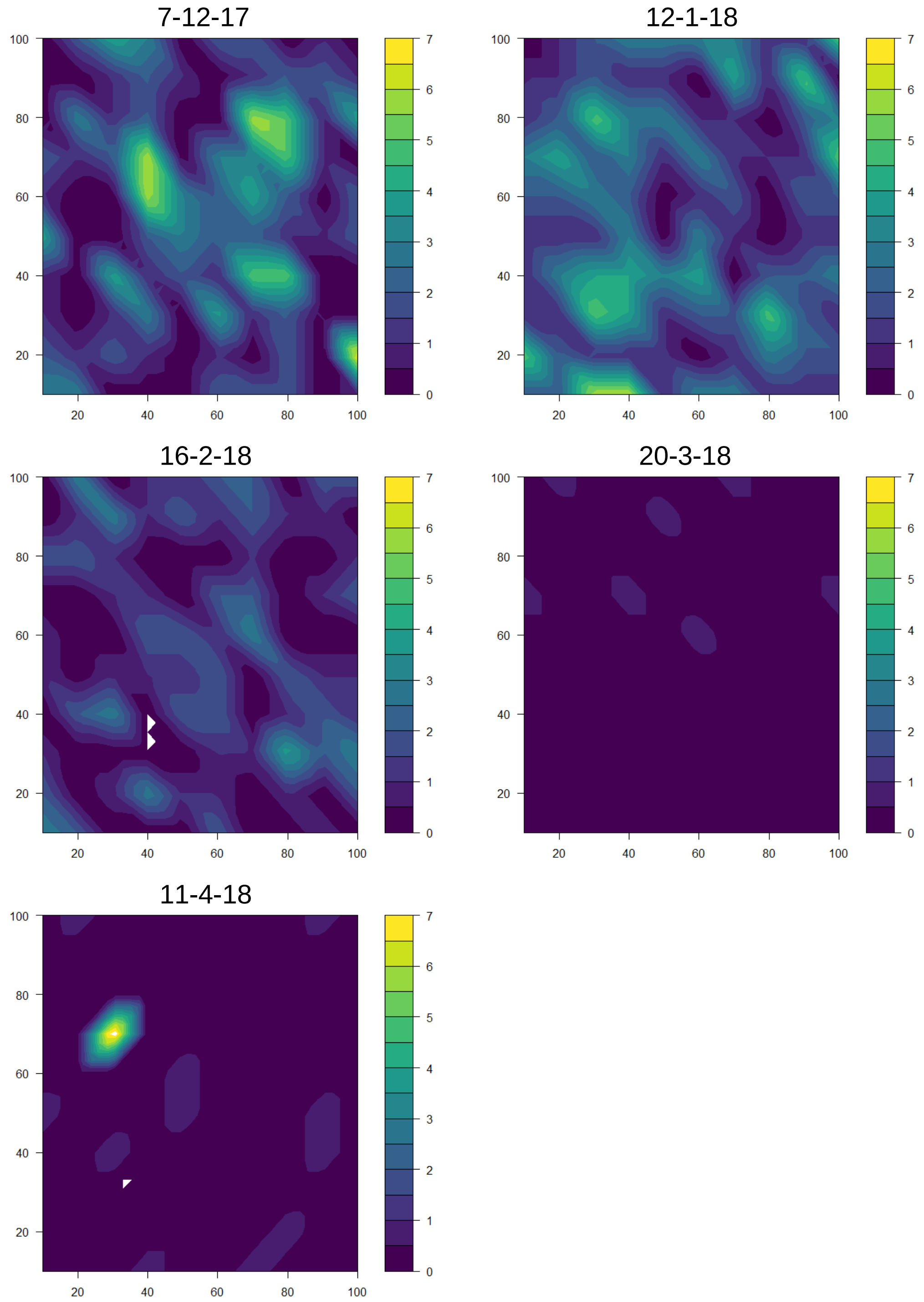


Figure 3.23. Heat maps showing slug distribution at Wigan from assessments carried out between December 2017 and April 2018. The numbers along the x and y axis show dimensions of the sampling grid in metres. 100 refuge traps were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the number of slugs, with the numbers in between traps calculated by polynomial interpolation.

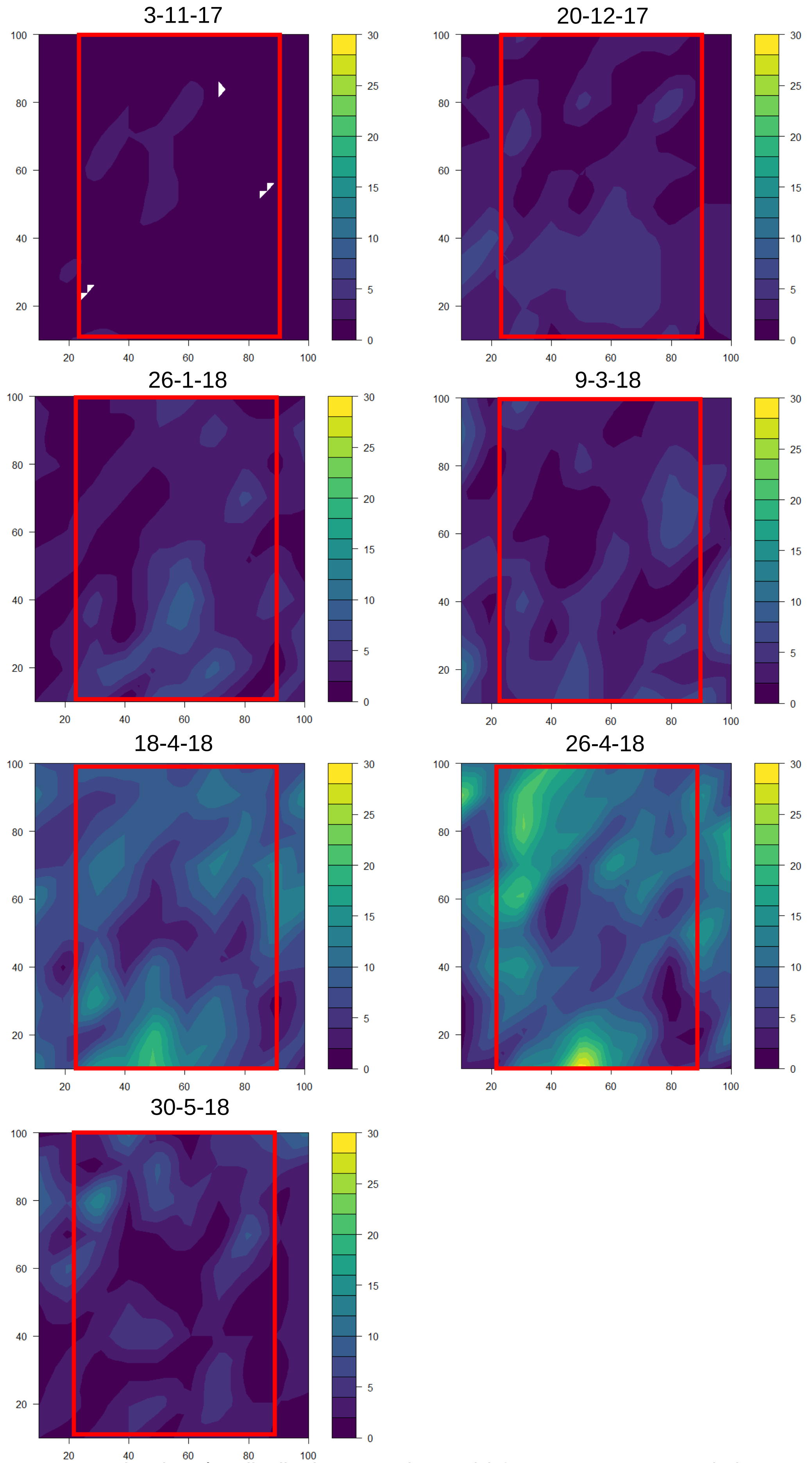


Figure 3.24. Heat maps showing slug distribution at Uppington (2) from assessments carried out between November 2017 and May 2018. The numbers along the x and y axis show dimensions of the sampling grid in metres. 100 refuge traps were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the number of slugs, with the numbers in between traps calculated by polynomial interpolation. The areas highlighted in red shows the location of the traps with the highest slug counts.3.3.4. Stability of patches between seasons

3.3.4. Between season stability of slug patches

Of the six fields where assessments were carried out in multiple years there were two fields, Uppington (1) and Adeney (Middle) where stable patches were detected in multiple seasons. In Uppington (1) the patches located in 2016-17 (Figure 3.17) were largely located in a different part of the grid to the patches located in 2015-16 (Figure 3.15). The correlation between counts in 2015-16 and 2016-17 at Uppington (1) was low (Table 3.110). There were only three significant correlations between seasons, two were positively correlated between 7-12-15 and 18-10-16 (r = 0.23, t=2.34, d.f.=98, p=0.021) and between 1-2-16 and 20-12-16 (r = 0.22, t=2018, d.f.=98, p=0.031)) and one was negatively correlated between 19-1-16 and 13-9-16 (r = -0.28, t=-2.93, d.f.=98, p=0.004) (Table 3.110)). Although patches were not detectable in Adeney (Middle) in 2016-17 or 2017-18 due to the low number of slugs recorded when the area of the field with the highest slug counts in 2015-16 (Figure 3.12) was compared with the 2016-17 and 2017-18 data on two out of nine and three out of eight assessments respectively the traps with the highest slug counts were located within the same area (Figure 3.25). The highest correlation was recorded between assessments on 18-1-16 and 7-3-17 (r = 0.33, t=3.50, d.f.=98, p<0.001; Table 3.10). In 2016-17 at Lynn (Stoney Lawn) patches were not detectable, however, on the assessment date with the highest individual trap count (10 on 16-12-16; Table 3.66), the area of the field with the highest number of slugs appeared in the same location as the highest trap counts in 2015-16 (Figure 3.144 and Figure 3.266). There were no significant correlations between the trap counts on the 16-12-16 and any of the assessments in the 2015-16 field season (Table 3.10). Weak correlations between counts on 24-5-16 and 11-1-17 and 15-3-17 and between 2-5-16 and 11-1-17 were observed (r=0.22, 0.22 and 0.27 respectively; Table 3.10). The data collected in this study do not provide sufficient evidence that the level of stability in the location of higher density slug patches over the life cycle of an individual crop (identified in earlier sections of this thesis), is reflected in similar stability between crops or cropping seasons.

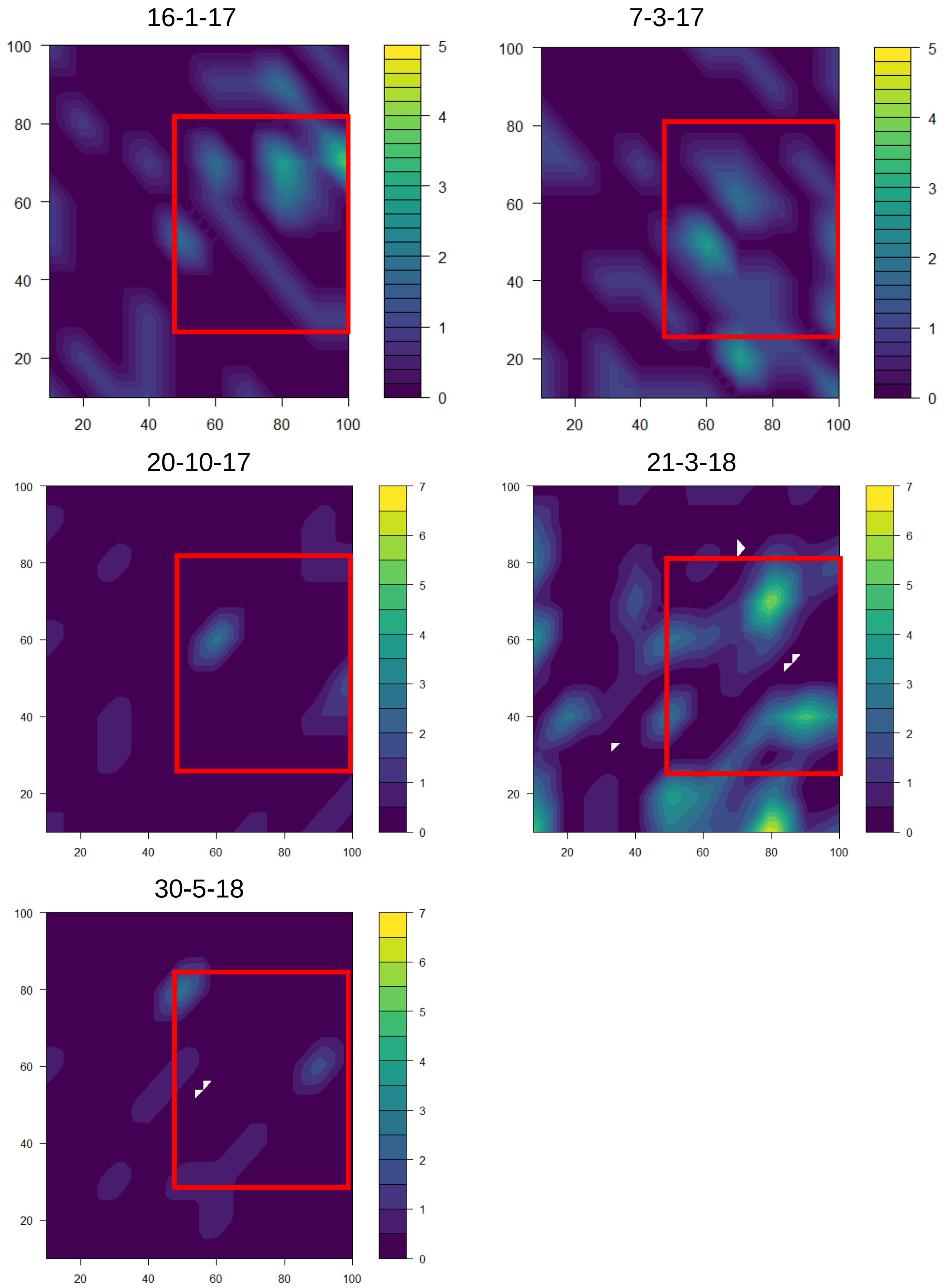


Figure 3.25. Heat maps showing slug distribution at Adeney (Middle) on 16-1-17, 7-3-17, 20-10-17, 21-3-18 and 30-5-18. The numbers along the x and y axis show dimensions of the sampling grid in metres. 100 refuge traps were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the number of slugs, with the numbers in between traps calculated by polynomial interpolation. The areas highlighted in red shows the location of the traps with the highest slug counts imposed from the 2015-16 season.

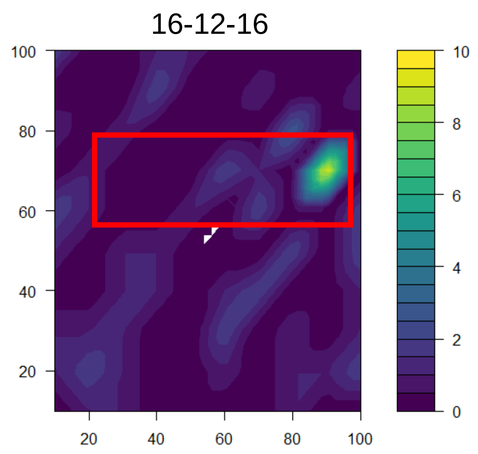


Figure 3.26. Heat map showing slug distribution at Lynn (Stoney Lawn) on 16-12-16. The numbers along the x and y axis show dimensions of the sampling grid in metres. 100 refuge traps were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the number of slugs, with the numbers in between traps calculated by polynomial interpolation. The areas highlighted in red shows the location of the traps with the highest slug counts imposed from the 2015-16 season.

Table 3.10. Pearson's Product Moment Correlation coefficient (r) between slug counts from 100 refuge traps in Adeney (Middle) on assessment dates between December 2015- April 2016 and January 2017- May 2018, Lynn (Stoney Lawn) on assessment dates between December 2015- May 2016 and December 2016 and Uppington (1) on assessment dates between December 2015-16 and September 2016- February 2017. Refuge traps were set up in a 10 by 10 grid at 10 metre intervals. Significant correlations between trap counts on different assessment dates are highlighted in yellow.

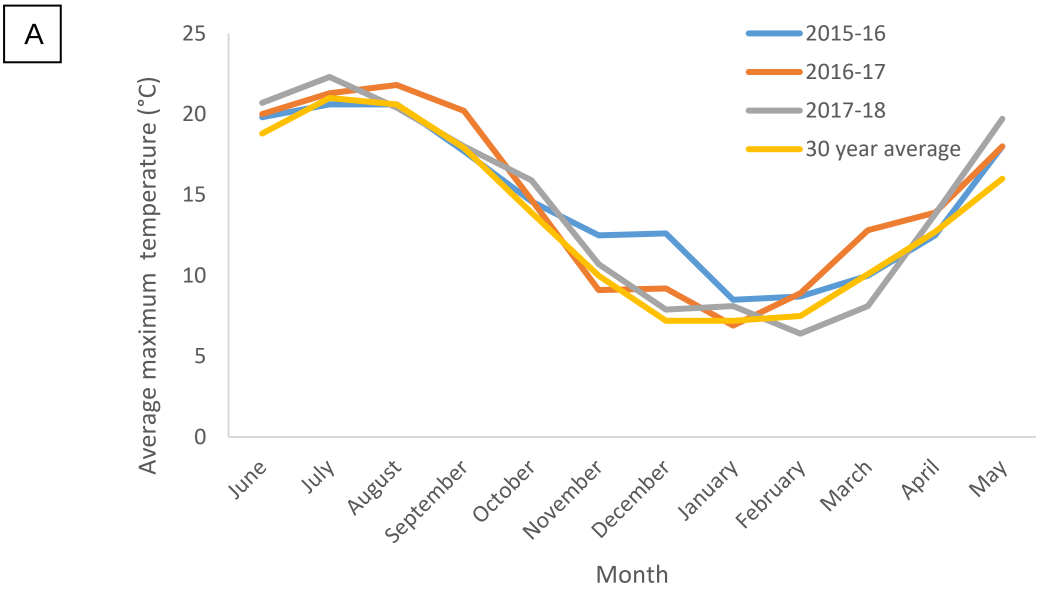
Adeney (Middle)	08/12/2015	22/12/2015	05/01/2016	11/01/2016	14/01/2016	18/01/2016	25/01/2016	29/01/2016	09/02/2016	12/02/2016	26/04/2016
16/01/2017	-0.05	-0.07	0.11	0.09	0.11	0.28	0.13	-0.11	0.19	0.16	0.17
07/03/2017	-0.28	0.07	0.21	0.21	0.23	0.33	0.14	0.11	0.24	0.14	-0.01
20/10/2017	-0.10	-0.07	0.02	-0.12	-0.08	-0.02	0.00	-0.01	0.07	0.20	0.02
21/03/2018	0.00	-0.22	0.13	0.05	-0.11	0.05	-0.05	-0.16	0.14	0.14	0.11
30/05/2018	-0.01	0.22	-0.14	0.20	0.01	0.05	-0.07	0.07	0.02	0.02	0.09

Lynn (Stoney Lawn)	07/12/2015	18/12/2015	22/12/2015	06/01/2016	11/01/2016	14/01/2016	21/01/2016	26/01/2016	02/02/2016	18/02/2016	15/03/2016	02/05/2016	24/05/2016
16/12/2016	0.01	-0.03	-0.09	-0.13	-0.04	-0.05	-0.07	-0.03	-0.03	0.00	-0.06	-0.03	-0.04
11/01/2017	-0.04	-0.06	-0.08	-0.02	-0.02	-0.05	-0.02	-0.04	-0.07	-0.14	-0.04	0.22	0.22
01/02/2017	0.19	0.07	0.03	0.04	0.12	0.14	0.10	0.07	0.09	0.17	0.13	-0.12	0.02
02/03/2017	-0.13	-0.02	0.02	-0.02	0.09	0.06	0.12	-0.01	-0.02	-0.04	0.09	0.00	0.10
15/03/2017	-0.02	0.07	0.06	0.00	0.10	0.00	0.05	0.12	0.02	0.02	0.05	0.05	0.27

Uppington (1)	13/09/2016	18/10/2016	23/11/2016	20/12/2016	17/01/2017	10/02/2017	28/02/2017
07/12/2015	0.11	0.23	0.01	-0.05	-0.07	-0.01	0.02
17/12/2015	-0.11	0.17	-0.02	-0.04	-0.09	-0.02	-0.08
04/01/2016	-0.14	0.11	0.12	-0.11	-0.03	0.10	-0.08
19/01/2016	-0.28	0.02	-0.05	-0.14	0.03	-0.15	-0.12
01/02/2016	0.03	0.16	0.19	0.22	0.11	-0.04	0.04
16/02/2016	0.06	-0.04	0.08	-0.08	-0.07	-0.05	0.06
29/04/2016	-0.05	0.05	-0.06	-0.10	-0.06	0.05	-0.09
23/05/2016	-0.18	0.06	0.05	0.05	-0.07	-0.04	-0.06

3.3.5. Environmental conditions

The weather in the three field seasons was variable. In 2015-16 the mean maximum and minimum air temperatures were higher in November (3.7 and 2.5°C respectively) and December (5.0 and 4.5°C respectively) than either of the other two field seasons and the 30-year average. Temperatures in March 2017 were also higher (mean maximum air temperature by 3.5°C and mean minimum air temperature by 2.5°C; Figure 3.27(A) and (B)). Differences in air temperatures were reflected in higher soil temperatures compared with the other field seasons in November and December 2015 and January 2016, there was no observable difference in soil temperature in 2017 as a result of the higher temperatures in March (Figure 3.27(C)). Rainfall was more variable (Figure 3.27(D)). Above average rainfall was recorded in eight of the twelve months between June and May in 2015-16 (difference >0.5 ml per day). In 2016-17 four of the twelve months had below average rainfall (including September, October, December and April) and in the 2017-18 field season there were six months with above (July, August, September, December, March and April) and two months with below average rainfall (October and May) (Figure 3.27(D)).



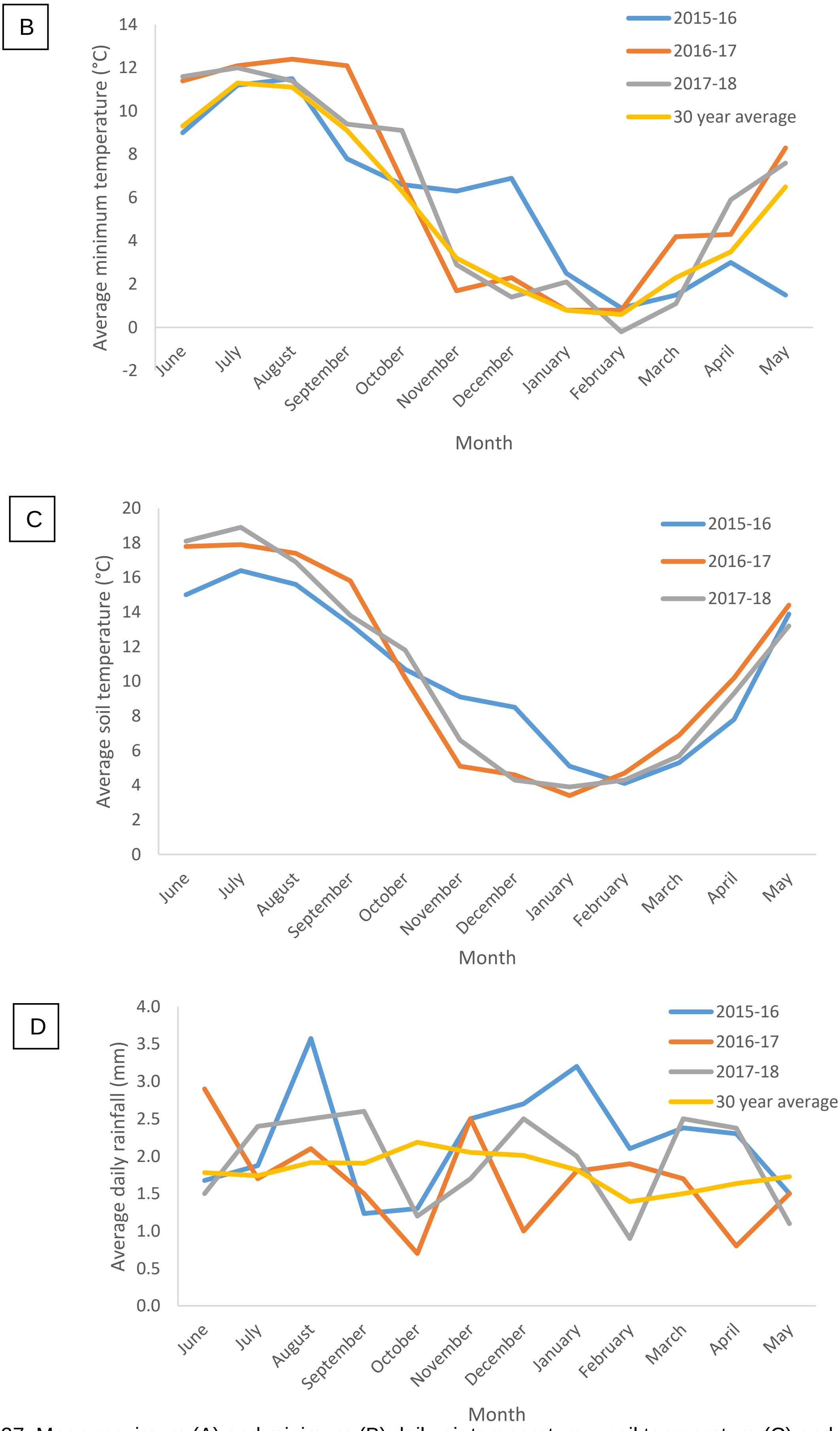


Figure 3.27. Mean maximum (A) and minimum (B) daily air temperatures, soil temperature (C) and daily rainfall (D) for each month during the 2015-16, 2016-7 and 2017-18 field seasons. Data collected from the Harper Adams University weather station. 30-year averages are also shown where data is available (MetOffice, 2019).

3.4. Discussion

Deroceras reticulatum can cause damage to a wide range of agricultural and horticultural crops, leading to significant economic losses (Nicholls, 2014; Twining *et al*., 2009). There is increasing pressure for growers to reduce pesticide usage and investigate alternative options of control. For example, the introduction of EU Directive 2009/128/EC requires National Action Plans for pesticide reduction to be established and along with the resulting UK Plant Protection Products (Sustainable Use) Regulations 2012 (PPP regulations) address the protection of watercourses and promotion of low input regimes, amongst other provisions (Defra, 2006). Non-uniform distribution of slug populations may offer the potential for reducing molluscicide use in agricultural fields. If areas of higher slug densities are found to be sufficiently spatially and temporally stable, and a commercially viable method of identifying their location and dimensions can be established without the need for refuge trap counts then control measures may be targeted at the patches, leaving areas with lower slug numbers untreated.

The species is known to display two peaks of reproductive activity in arable fields, in the spring and autumn (Port & Port, 1986) which were observed in this study. Raised slug activity during autumn was reflected in refuge trap catches between November and December and a second, higher peak was recorded between late February and May. Thereafter catches remained low until the winter wheat crops were harvested in August. Slug numbers varied widely between fields with some exceeding recommended treatment thresholds (AHDB, 2016).

The discontinuous distribution of slugs in arable fields reported by Bohan *et al*. (2000a) and Archard *et al*. (2004) resulted in patches of higher slug numbers interspersed within areas of lower slug densities being readily detected in all fields investigated in this study, even when different environmental conditions prevailed. The first hypothesis “Clear areas with high slug densities can be identified and defined within arable fields in the UK” is upheld by this study. Refuge traps were used to assess slug activity on the soil surface where factors such as soil moisture and temperature, which vary throughout the growing season, can influence their behaviour (Choi *et al*., 2004). During periods of sub-optimal physical conditions, a significant proportion of the population retreats to a protected environment, below the soil surface (South, 1992) where they cannot be detected using refuge traps. Consequently, the patches of high slug population densities were not recorded at every sampling visit, but critically within a cropping season were located in the same areas of the field when they were detected, suggesting that slugs either move vertically between the upper soil horizon and the soil surface, or return to the same locations in the field when conditions are favourable. Understanding the mobility of slugs will be key to understanding the mechanisms underpinning the formation of population patches and thus their temporal stability. Successful suppression of slug populations following application of slug pellets may have contributed to masking of the location of the field patches prone to harbouring higher slug numbers by significantly lowering trap catches across the field (including within the patches). Critically, however, as patches subsequently reappeared in the same areas when slug surface activity increased again, their location is likely to be dependent on spatially stable environmental characteristics (Carrick, 1942; Ondina *et al.*, 2004). Where slug numbers were sufficient, within-season patch stability was high in all three growing seasons, confirming the second hypothesis "The areas of high slug densities identified are sufficiently spatially and temporally stable to facilitate the targeted application of controls". The proposition that molluscicide use in agricultural fields may be reduced by targeted application of control measures to areas of high slug densities within fields is dependent on a cost-effective method of defining patch location at or before sowing of the crop can be developed. The size of patches identified in this study varied between 300 and 7000 m^2, which would be suitable for molluscicide application using current technology. In a test of slug pelleters the spread width of pellets was found to be between 12 and 30 m (Mark, 2014), supporting the third hypothesis "The individual and cumulative size of the slug patches would allow the efficient targeting of control treatments".

Slug activity is known to be dependent on environmental conditions such as temperature and rainfall (Choi *et al*., 2004), the large variations in the number of slugs detected between years, could be a result of the variation in temperature and rainfall between years. November and December 2015-16 were mild compared to the following two years, the average minimum temperatures in November were 4.6°C and 3.4°C warmer than in 2016 and 2017 respectively and 3.1°C warmer than the 30-year average (MetOffice, 2018). Unlike other slug species which synchronise their development with photoperiod *D. reticulatum* will reproduce when conditions are favourable i.e. mild and damp and juveniles can continue to develop throughout the winter period, although the rate of development of eggs and juveniles is affected by temperature (South, 1982), contributing to the higher numbers of slugs observed in the milder 2015-16 season than in the colder 2016-17 and 2017-18 seasons.

Between season patch stability was not confirmed in this study, with limited data for multiple field seasons due to the low number of slugs detected in some fields. The data available makes a clear conclusion on patch stability between seasons difficult. The areas of higher density patches occurring in different areas of the grid in Uppington (1) in the 2015-16 season and 2016-17. The low number of slugs in Adeney (Middle) during the 2016-17 and 2017-18 field seasons meant detection of stable patches was not possible, however, when the location of the patch in 2015-16 was imposed on the heat maps for the following two seasons the highest number of slugs occurred in that area on two out of nine and three out of eight of assessments. The highest count in Lynn (Stoney Lawn) in the 2016-17 season occurred in the same area of the field as the patch location in 2015-16. Further work is required looking at multiple seasons with high slug counts to enable a firm conclusion to be made on patch stability between seasons. The effect of cultivations on slug distributions requires also warrants further investigation as there is evidence that movement of the soil may disrupt the distribution (Glen *et al.,* 2006a). If patch stability between years does occur, then the cost of defining patch location would be reduced as the frequency of measurements would be lower and the initial cost could be shared over more than one year making any effective patch location method more economically viable.

This study suggests that there is the potential for slug patches to be sufficiently temporally and spatially stable within field seasons for targeted application of molluscicides. Further work is required to confirm long term stability of patches over multiple growing seasons. In order to overcome the commercial restrictions of low trap counts early in the season and the large number of refuge traps that were utilised in this research, alternative methods of locating the higher density patches will be investigated. Alternative methods of identifying the location of areas of higher slug densities within fields which are not reliant on surface activity are discussed further in the following chapters. These methods include use of damage as an indicator of patch location as well as soil characteristics.

3.5. Conclusion

Areas of high slug densities were found to be sufficiently stable throughout a growing season to allow the possibility of targeting molluscicide applications. In fields where slug numbers were high the correlation between trap counts were high throughout the season, where slug numbers were low the variation between trap counts was insufficient for identifying patches. Variations in environmental conditions resulted in differences in slug abundance between seasons, however, similar patterns were present in each of the seasons studied. No conclusive evidence for between season patch stability was found in this study.

Chapter 4. Assessing the potential of damage assessments to locate areas of higher slug densities

In Chapter 3 slug patches identified in the study fields were shown to be stable over long periods of time (within a cropping season). This is an important characteristic if treatment applications solely targeting areas with high slug densities are to be developed as a viable management option. The need for an alternative method of locating slug patches has been introduced in previous chapters. Anecdotal evidence from growers and agronomists suggests that areas of damage can be used to identify where the highest slug numbers occur. This chapter investigates the potential for damage assessments to be used as a practical tool for locating areas of higher slug densities in arable fields, which can be used to inform slug management decisions. The 10 by 10 grid introduced in Chapter 2 will be used to relate damage to slug counts.

4.1. Introduction

4.1.1. Current method of pellet application decisions

Current recommendations for cost effective molluscicide applications are dependent on refuge trap counts in conjunction with thresholds. Nine refuges (or 13 in fields over 20 ha), consisting of a 25 cm diameter plant pot saucer baited with chicken layers mash are set in each field in a ‘W’ pattern. Assessments are targeted to periods when the soil surface is moist and temperatures are between 5 and 25°C. Management guidelines state that refuge traps should be left overnight and the number of slugs using each trap recorded before temperatures rise and slugs leave the traps. The guidelines, however, do not specify what constitutes a temperature rise. The threshold for pellet application depends on the crop grown (Table 4.1; AHDB, 2016), with current recommendations that pellets are applied across the whole field where these levels are met or exceeded. Chapter 3 demonstrated that slugs are not uniformly distributed across fields, and therefore, random placement of traps in a ‘W’ pattern across a field could lead inaccurate assessment of population levels. Petrovskaya *et al*. (2018) investigated the effect of reducing the frequency of traps and concluded that coarse trapping, in this case four traps per hectare, a lower density than currently recommended, would lead to unreliable population estimates. Consequently, a new method of locating patches needs to be developed to allow more reliable targeting of controls.

Table 4.1. Action thresholds for the application of control measures for slugs in major field crops (AHDB, 2016)

Crop	Threshold (mean number of slugs/trap)
Winter cereal	4
Oilseed rape (standing cereal)	4
Oilseed rape (cereal stubble)	1
Potatoes	1
Field vegetables	1

4.1.2. Variation in surface activity due to weather conditions

One of the factors contributing to the low uptake of the current recommendations for commercial slug assessments and thresholds is the variability of slug surface activity related to different weather conditions (Choi *et al.,* 2004), which may result in the refuge trap approach being unreliable. It has been suggested that alternative methods of locating patches, which avoid such variation due to changing weather conditions could potentially include using damage assessments or soil characteristics to identify areas of the field with higher slug densities.

4.1.3. Current precision farming practices

There are several existing examples of precision application methods where inputs, such as pesticides, fertiliser applications or irrigation, are not uniform across the field and are tailored to the requirements of different areas (Lindblom *et al.,* 2017). There are alternative ways of detecting the varying requirements across fields, including in-field moisture sensors that vary the rate of irrigation to meet demand (Haghverdi *et al.,* 2015) and thermal imaging drones informing fungicide application rates such as for *Fusarium* head blight in wheat (Mahlein *et al.,* 2012). Currently the majority of targeted pesticide applications are for fungal related diseases such as take-all, powdery mildew and *Septoria* (Wójtowicz *et al.,* 2016), whereby fungicides are applied to specific problem areas of the crop (Tackenberg *et al.,* 2018). Increasingly the potential for precision targeting of insecticides is being investigated, for example targeted application of seed treatments for cabbage stem flea beetle (Sekulic and Rempel, 2016) and targeting pesticides for cotton fleahopper, verde plant bug and sugarcane aphids (Deleon *et al.,* 2017).

4.1.4. Discontinuous distributions offer potential for targeting pesticides

Ferguson *et al*. (2003) demonstrated the potential for targeting insecticides in OSR for the control of cabbage shoot weevils, cabbage stem weevils, pollen beetle and brassica pod midge. Insects were found to be aggregated within OSR crops and these non-uniform distributions were linked to variation within the crop of seed loss, pod splitting, bud abscission and the rate of plant maturation, all of which will negatively affect the final yield.

They concluded that in order for insect populations to be targeted it is important that the interaction between insect behavioural and environmental factors which determine the distribution are properly understood before precision application of pesticides can be used reliably.

4.1.5. Alternative methods of locating slug populations

Methods of relating the location of slug patches to other factors in the field have been investigated by Bohan *et al.* (2000b) who related the distribution of slugs to carabid beetle activity as a basis for conservation biocontrol strategies. In this study destructive soil samples were taken to measure slug abundance, which meant that the grid for mapping slug populations was offset by 2.5 m from the grid for measuring beetle populations and the position of the grid had to move by 1.5 m on each sampling date to avoid the area where the soil had previously been removed. This technique allowed a comparison of slug and beetle populations on two sampling visits but the destructive nature of the sampling would not support long term studies. Mueller-Warrant *et al.* (2014) investigated slug numbers in relation to damage in Oregon, USA and found a weak correlation between slug counts and damage in clover fields, using a minimum of 30 slug blankets per field spaced at one blanket per acre. Counts of slugs were carried out weekly over a 19-week period and the percentage loss of crop stand was. Using non-destructive surface refuge traps the authors were able to repeatedly sample the slug distribution over time, however, this paper does not provide details of the frequency of crop assessments or the proximity to the slug counts. Literature sources (Bohan *et al.,* 2000a; Glen *et al.,* 2003) suggest that refuge traps would need to be positioned on a finer grid than that used by Mueller-Warrant *et al.* (2014) to obtain accurate assessments of the slug spatial distribution and damage assessments would need to be carried out in close proximity to the refuge traps.

4.1.6. Slug damage in wheat

Slugs cause damage to wheat crops primarily by seed hollowing, with the level of damage being dependent on seed depth at drilling, and the condition of the seed bed. Cloddy seed beds will allow slugs easy access to the seeds compared to a fine seed bed, and shallow drilled seed is more susceptible to slug damage than deeper drilled seed (Glen *et al.,* 1990b). After germination seedlings are also vulnerable to slug damage by leaf shredding until GS21 (main shoot and one tiller, AHDB, 2016). Crops are able to compensate for low levels of damage but even at low levels of damage slugs can be economically damaging (Table 4.2). It is questionable whether at the early growth stages the relatively low, but still economically important, levels of damage would be sufficient to identify patches of crop which would need to be treated.

Table 4.2. Percentage plant and yield losses following different levels of simulated slug damage. Losses are expressed as a % reduction in treatment plots with simulated slug damage compared to untreated control plots (Jessop, 1969).

Simulated damage (%)	Plant loss (%)	Yield loss (%)
25	20	4
50	65	19
92	82	34

4.1.7. Objectives and hypotheses

This chapter aims to draw conclusions on the potential for using damage assessments as a practical tool by investigating whether patches of higher slug density (that persist in a stable location throughout the growing season) can be identified in commercial winter wheat fields from plant damage caused by slug feeding alone.

Objectives

- To identify the location of the higher density slug patches using the standard 10 by 10 grid.
- To determine whether the distribution of plant damage caused by slug feeding is related to slug catches recorded in refuge traps.set in the sampling grid.

Hypotheses

- Crop patches which contain higher slug numbers can be identified in winter wheat fields using the standard trapping grid described in Chapter 2.
- Plant damage caused by slug feeding within 50cm of refuge traps set in the standard trapping grid is consistently and significantly related to the number of slugs caught in those traps and supports the use of plant damage as a predictor of slug patch location in commercial practice.

4.2. Materials and Methods

The standard experimental grid developed in Chapter 2 (10 by 10 traps with 10 m intervals between nearest traps) was established in five commercial fields in Shropshire, UK each sown with winter wheat following a previous crop of OSR (Adeney (Middle), 52°46'2.535"N -2° 26' 38.85"E, cv. Reflection; Adeney (Corner), 52°45'56.9268"N -2°26'40.4736"E, cv. JB Diego; Lynn (Badjics), 52°43'44.8746"N -2° 20' 11.8392"E, cv. Reflection; Lynn (Stoney Lawn), 52°44'11.9112"N -2°21'2.2818"E, cv. Reflection, Uppington (1), 52°40'37.0848"N -2°34'49.296"E, cv. Horatio). All fields were cultivated using a subsoiler and disc harrow followed by rolling. At each site crop husbandry followed normal farm practice with between 1 and 3 applications of molluscicide.

4.2.1. Experimental design and slug assessments

Grids were established at a minimum of 20 m from the nearest field edge and with the nearest edge of the grid parallel to the field edge. The number of slugs under each refuge trap was counted at approximately 14-day intervals between week commencing 30 November 2015 (week 1) and 15 February 2016 (week 12), and thereafter monthly until week commencing 23 May 2016 (week 26). These counts were used to investigate the relationship between slug numbers and crop damage.

4.2.2. Damage assessments

The percentage leaf area damaged by slugs (slug damage identified following the definitions of AHDB (2014)) was recorded from 20 haphazardly selected leaves located within a circle (50 cm radius) centred on each refuge trap. The mean leaf damage was calculated for each trap at each sampling visit. Sampling for damage levels was extended beyond the period in which slug controls might usually be applied to test the relationship between damage and a wider range of slug populations, as reflected by the catches of the surface refuge traps.

4.2.3. Analysis of slug distribution and crop damage

Maps of slug numbers and damage distributions were produced using the interp function of R version 3.3.1. (R Core Team, 2013), a polynomial interpolation between the grid nodes. Hotspot analysis was used to identify areas of the field with significantly higher numbers of slugs than would be expected in a random distribution. The correlation between trap counts on different dates, and between trap counts and leaf damage assessments at each trapping point were quantified using Pearson's Product Moment correlation coefficient *r*. Statistical analysis comparing slug population size in different fields was conducted using analysis of variance, post hoc Tukey’s HSD tests were carried out to identify where significant differences were occurring.

4.3. Results

4.3.1. Deroceras reticulatum populations

The number of slugs recorded in refuge traps varied both between assessment visits within fields and between different fields during the cropping season ($F=12.10$, d.f.=4,41, $p<0.001$. Post hoc (Tukey’s HSD) analysis showed that the significant difference between fields was due to the higher numbers recorded at the Lynn (Stoney Lawn) field site (833.8 ±129.4). No significant differences were detected between the mean of the total number of slugs recorded in the sampling grid at each visit during this period at Uppington, Adeney (Middle), Adeney (Corner) and Lynn (Badjics) (Table 4.3).

Slug numbers varied with time in the winter wheat fields studied (Figure 4.1). Significantly lower numbers of slugs were recorded between 30 November and 22 December 2015 ($F=6.47$, d.f.=1,40, $p=0.015$), with a mean trap count of 204.6 ±44.4 slugs per trap on each assessment date compared to 451.6 ±72.2 for the period 4 January to 29 April 2016. Post hoc (Tukey's HSD) analysis showed slug numbers at Lynn (Stoney Lawn) were higher during the period 4 January to 29 April 2016 (Figure 4.1). The mean total number of slugs recorded in the sampling grid at each assessment between 4 January and 29 April 2016, was 257.5 ±94.8 at Uppington and corresponding figures of 319.7 ± 47.8 at Adeney (Middle), 284.2 ±42.1 at Lynn (Badjics), and 960.0 ±145.4 at Lynn (Stoney Lawn). Slug populations in Adeney (Corner) were not significantly different from Uppington, Adeney (Middle) and Lynn (Badjics) but did not follow the pattern of the other fields and remained low throughout this period with a mean of 72.8 ±18.3 slugs per assessment during this later period. Within each field, the maximum number of slugs recorded during an individual assessment visit was 663 at Uppington (week 22, 29 April), with corresponding maxima at Adeney (Middle) of 667 (week 22, 26 April), Lynn (Badjics) of 400 (week 12, 18 February), Lynn (Stoney Lawn) of 1796 (week 16, 15 March) and Adeney (Corner) of only 133 (week 5, 5 January). Due to the low number of slugs recorded in Adeney (Corner), no further analysis of the data collected was undertaken.

Table 4.3. The mean (±SE) number of slugs in Adeney (Middle), Adeney (Corner), Lynn (Badjics), Lynn (Stoney Lawn) and Uppington (1). Where letters differ significant differences occur (ANOVA and post-hoc Tukey's HSD).

Field	Mean number of slugs per assessment 30 November 2015 – 22 December 2015	Mean number of slugs per assessment 4 January 2016 – 29 April 2016	Maximum number of slugs
Adeney (Middle)	144.0 ±24 a	319.7 ±47.8 a	667
Adeney (Corner)	47.0 ± 22 a	72.8 ±18.3 a	133
Lynn (Badjics)	158.0 ±3 a	284.2 ±42.1 a	400
Lynn (Stoney Lawn)	412.0 ±55.7 b	960.0 ±145.4 b	1796
Uppington (1)	156.5 ±8.5 a	257.5 ±94.8 a	663

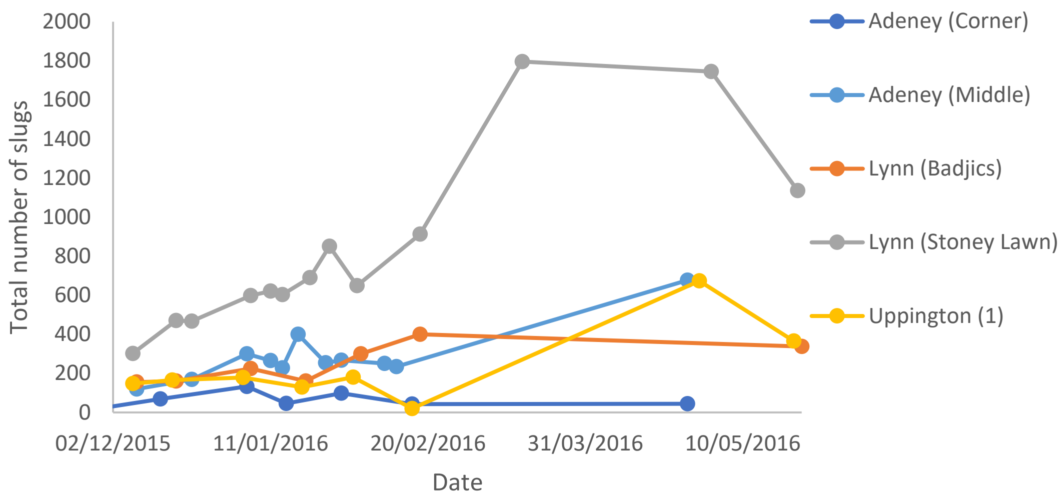


Figure 4.1. The total number of slugs on each assessment date throughout the 2015-16 growing season. Slug counts were carried out in five winter wheat fields in Shropshire, UK using a 10 by 10 grid of refuge traps placed at 10 metre intervals.

Irrespective of the variable population sizes of slugs in different fields, hotspot analysis detected discrete areas of higher slug densities in all fields investigated (Figure 4.2). Variation between assessment visits in numbers of slugs that were active on the soil surface (reflected in refuge trap records) resulted in a corresponding disappearance of slug patches when very low catches were recorded and their reappearance in the same area of the field as trap catches increased (Figure 4.3).

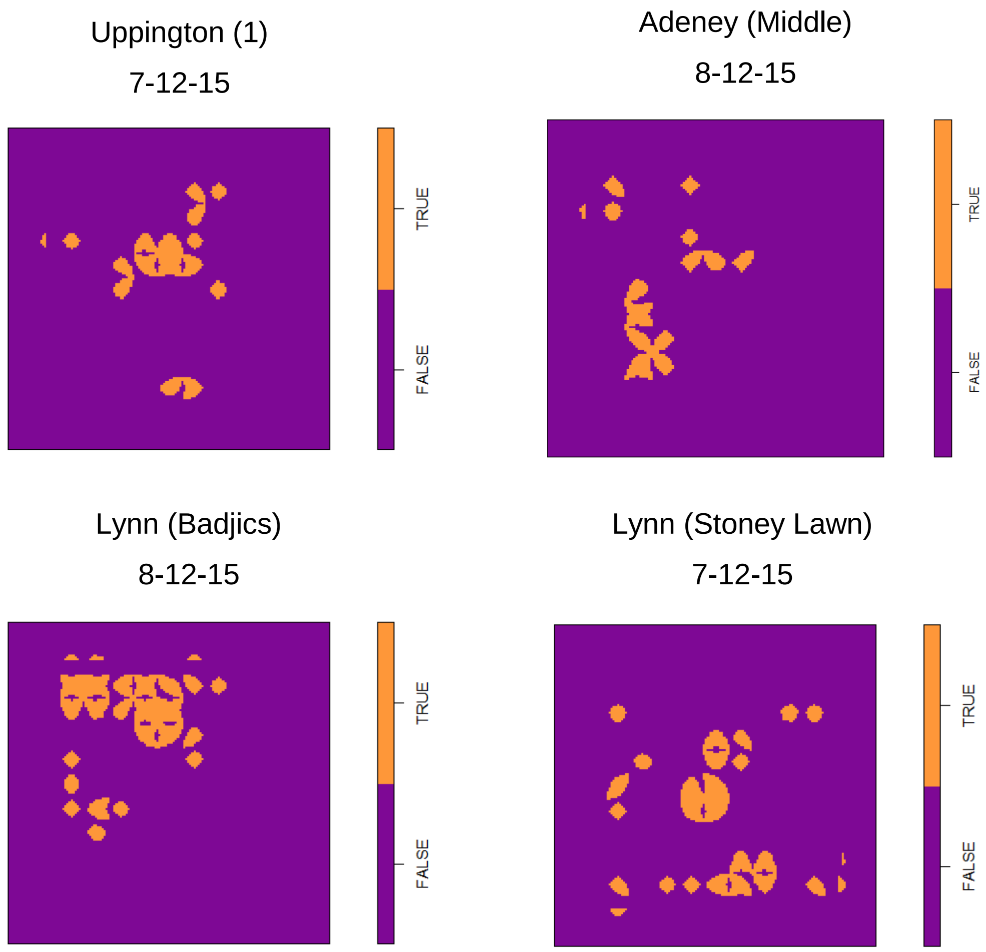


Figure 4.2. Hotspot analysis confirming the discontinuous distribution of *Deroceras reticulatum* in four commercial winter wheat fields in Shropshire, UK, sampled in week 2 of the study (7 to 13 December 2015). The area represents a 100 x 100 metre trapping grid used in the study in which 100 refuge traps were positioned at 10 metre intervals in a 10 by 10 grid. Orange represents significant aggregations of slugs (areas where more slugs were found than would be expected if the slugs were randomly distributed across the field at $p<0.05$ significance level). Purple shows the areas of the field where the number of slugs observed was not significantly different to that expected if they were randomly distributed.

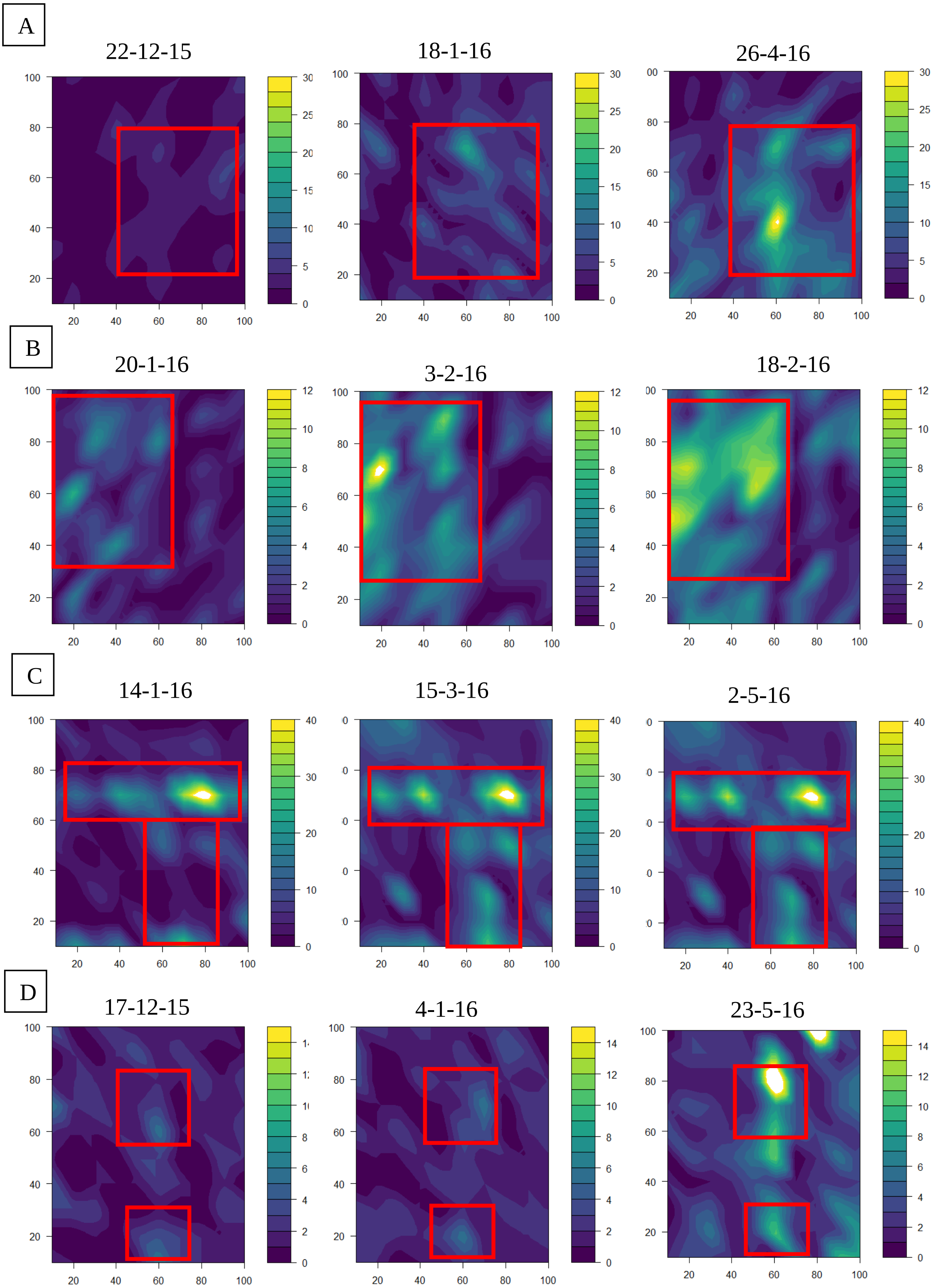


Figure 4.3. Heat maps showing slug distribution at (A) Adeney (Middle), (B) Lynn (Badjics), (C) Lynn (Stoney Lawn) and (D) Uppington (1) from assessments carried out between December 2015 and May 2016. The numbers along the x and y axis show dimensions of the sampling grid in metres. 100 refuge traps were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the number of slugs, numbers in areas between traps were calculated by polynomial interpolation. The areas highlighted by red boxes show the location of the traps with the highest slug counts.

4.3.2. Correlation between location of damage and slug counts

Slug feeding damage varied significantly between fields ($F=8.90$, d.f.=3,25, $p<0.001$). Post hoc (Tukey's HSD) analysis showed the field with the highest percentage leaf area damaged was recorded in Lynn (Stoney Lawn) (Figure 4.4), reflecting the high slug numbers recorded in refuge traps. No significant differences in percentage leaf damage occurred between any of the other fields assessed. Damage scores decreased in all fields over the growing season reflecting the increase in plant size (Figure 4.4; 4.5; 4.6; 4.7; 4.8), whereas the number of slugs per trap increased throughout the season (Figure 4.1).

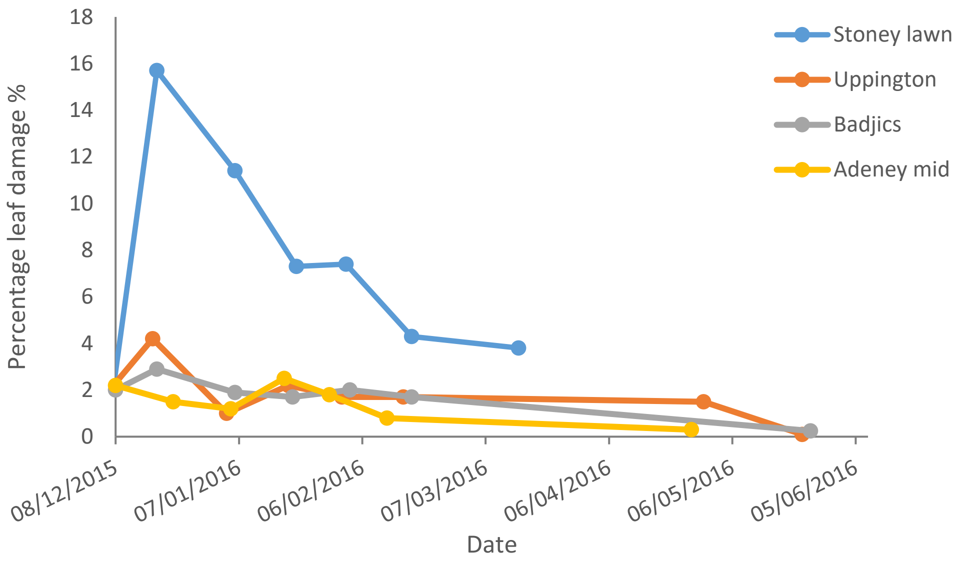


Figure 4.4. Mean percentage leaf area damaged on each assessment date between December 2015 and May 2016 at each field site. Damage was assessed as a percentage leaf damage of 20 leaves within a 50 cm radius of each refuge trap across a 10 by 10 grid.

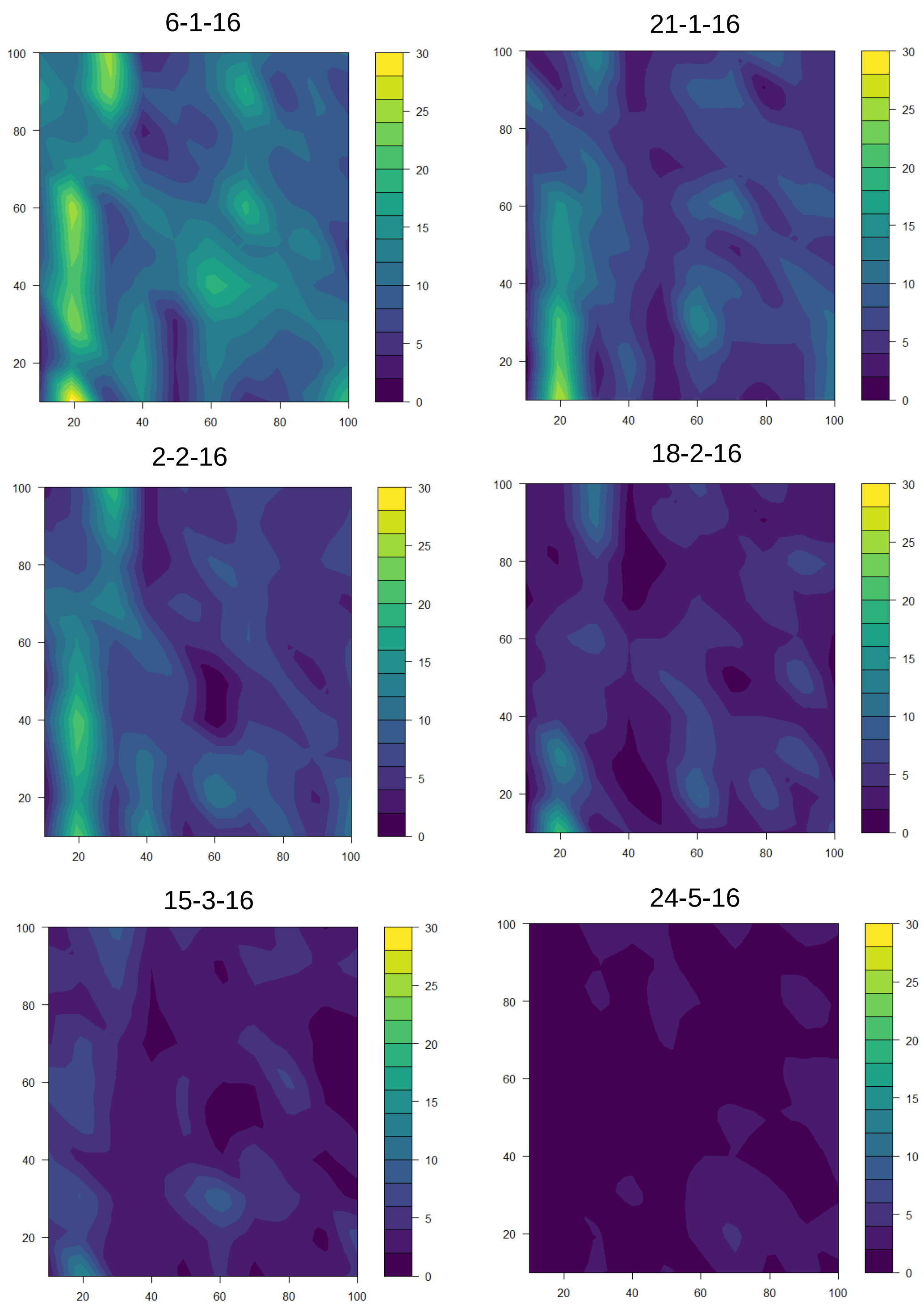


Figure 4.5. Heat maps showing the distribution of damage caused by *Deroceras reticulatum* feeding in a commercial winter wheat field, Lynn (Stoney Lawn) between January and May 2016. The numbers along the x and y axis show distance in metres. Sampling points were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the percentage leaf area damaged; damage levels in areas between sampling points were calculated using polynomial interpolation.

Significant correlations between the percentage feeding damage recorded on plants and slug catches in refuge traps at each grid point were found in each field. At Lynn (Stoney Lawn), the field with the highest number of slugs, there was one significant correlation on 2/2/16 (Table 4.4), which was negative. The correlations between damage and slug numbers were positive in the other three fields; in 6 out of 7 assessments at Adeney (Middle), six out of seven assessments at Lynn (Badjics) and three out of eight assessments at Uppington (1) (Table 4.4; Figure 4.6; 4.7; 4.8). Pearson's Correlation

Coefficients at Adeney (Middle) varied between r = 0.24 (26/4/16) and r = 0.43 (29/1/16), at Lynn (Badjics) between r = 0.23 (6/1/16) and r = 0.52 (18/12/15) and at Uppington (1) between r = 0.18 (7/12/15) and r = 0.52 (17/12/15). Although statistically significant the correlations between the damage assessments and slug counts were weak (r < 0.39) or moderate (r = 0.40-0.59) and no positive correlation was found at Lynn (Stoney Lawn) where the field had the highest number of slugs. The weak relationship between apparent slug damage and numbers of slugs caught in refuge traps suggests that visible slug damage is a poor indicator of the location of patches of higher slug densities, even in winter wheat fields with higher slug populations.

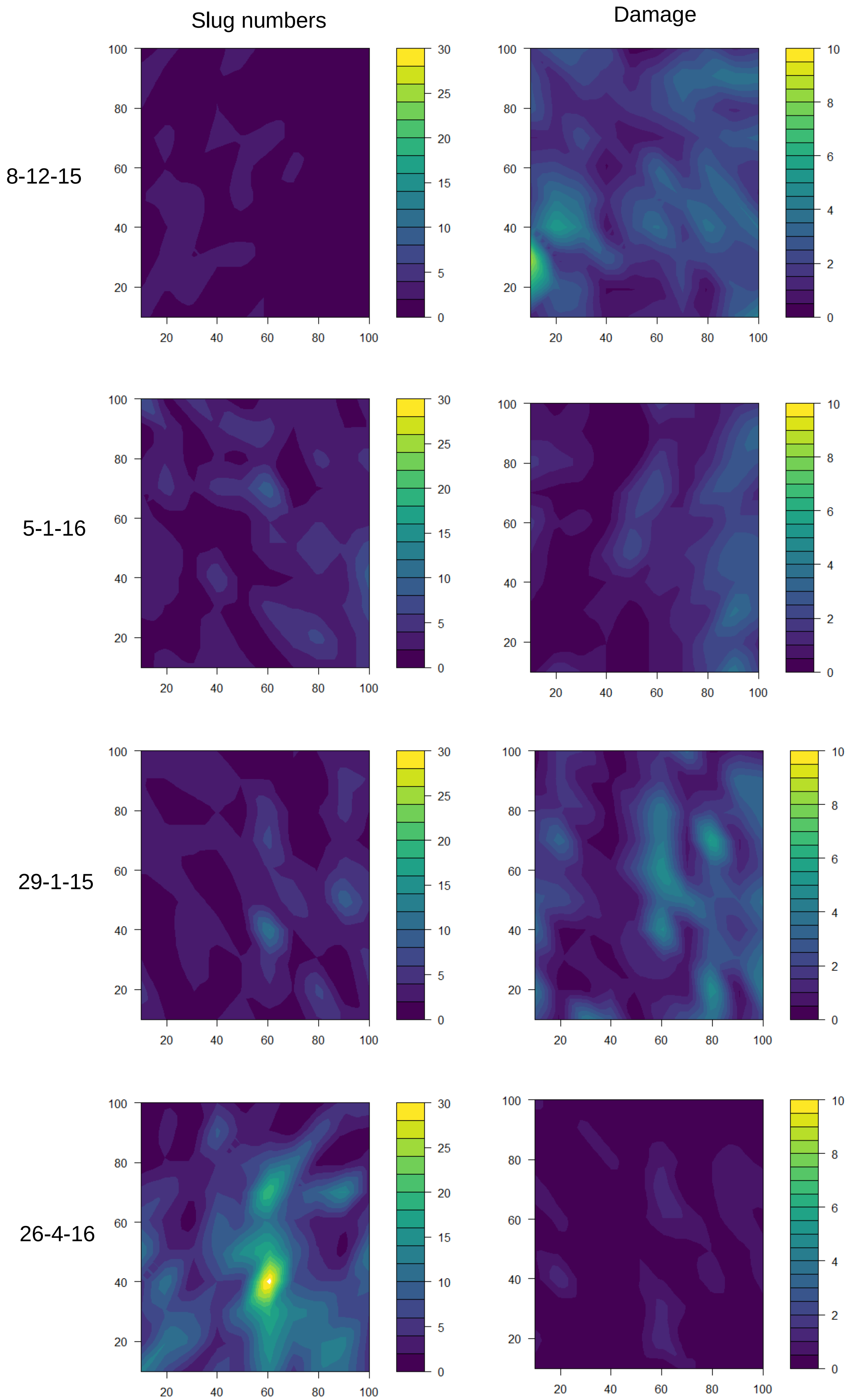
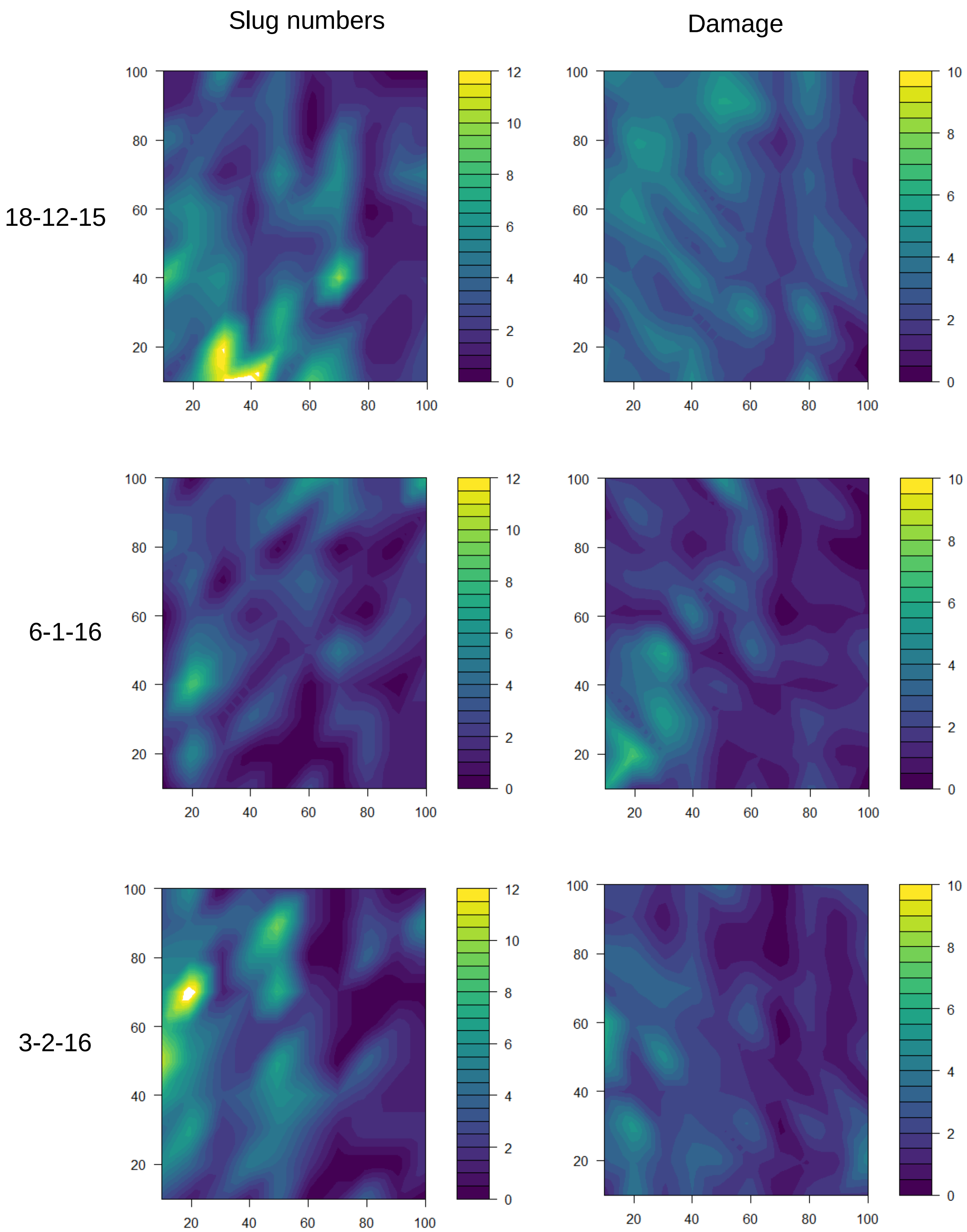


Figure 4.6. Heat maps of slug distributions and percentage leaf damage at Adeney (Middle) on assessments between December 2015 and April 2016. The numbers along the x and y axis show distance in metres. Sampling points were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the number of slugs and percentage damage, with the numbers in between calculated by polynomial interpolation. Damage assessments were carried out on 20 leaves in a 50 cm radius of each refuge trap.


Slug numbers
Damage
18-12-15
6-1-16
3-2-16

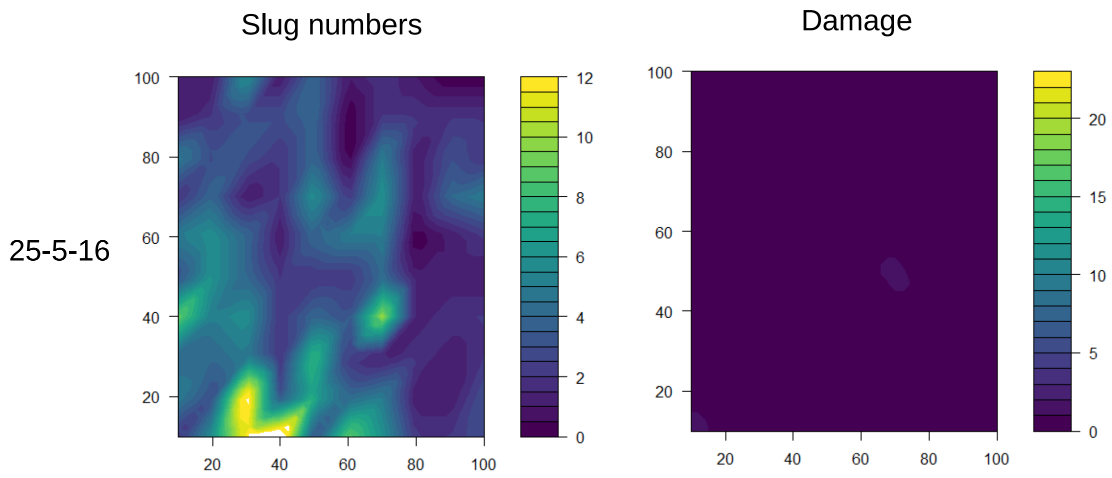


Figure 4.7. Heat maps of slug distributions and percentage leaf damage at Lynn (Badjics) on assessments between December 2015 and May 2016. The numbers along the x and y axis show distance in metres. Sampling points were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the number of slugs and percentage damage, with the numbers in between calculated by polynomial interpolation. Damage assessments were carried out on 20 leaves in a 50 cm radius of each refuge trap.

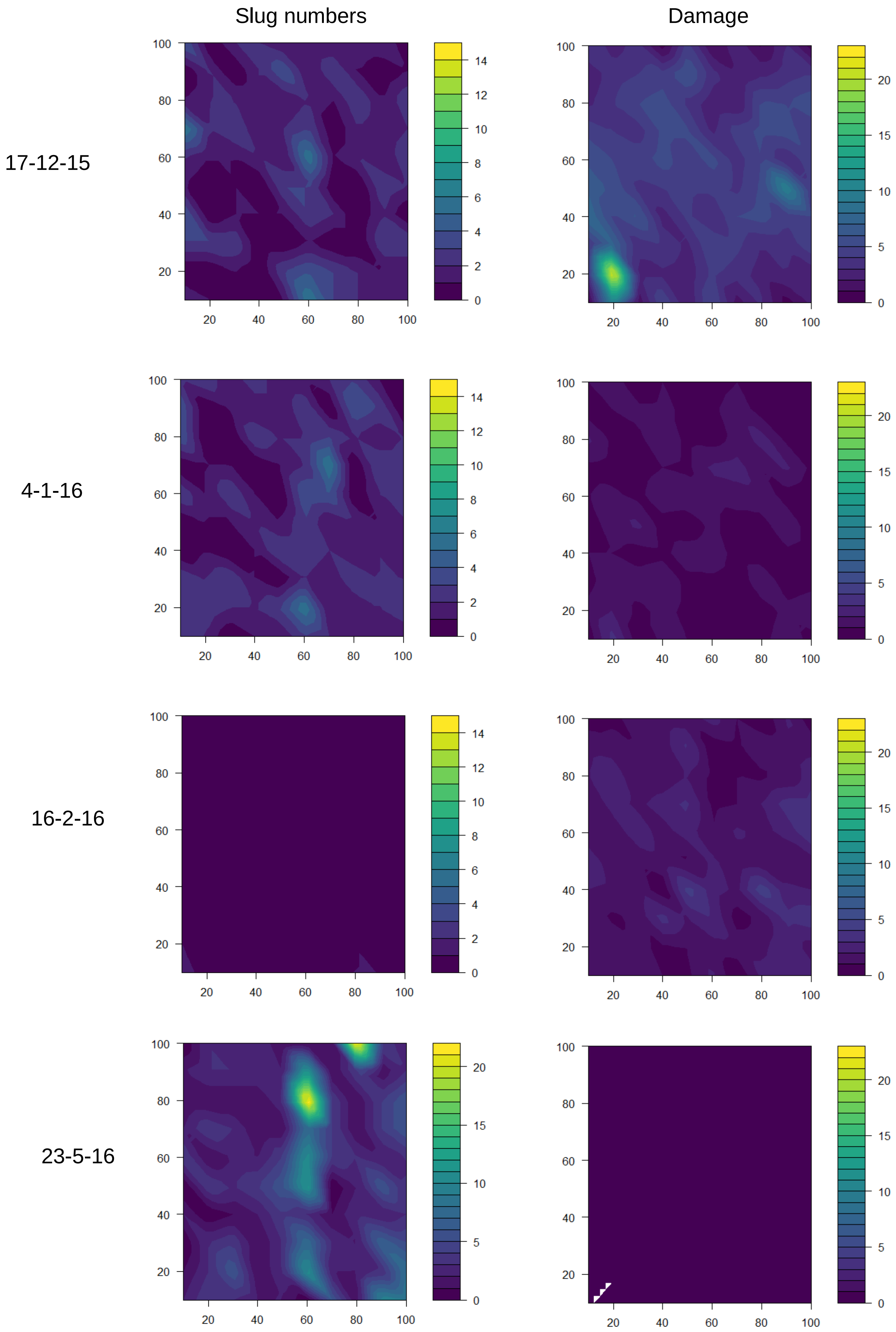


Figure 4.8. Heat maps of slug distributions and percentage leaf damage at Uppington (1) on assessments between December 2015 and May 2016. The numbers along the x and y axis show distance in metres. Sampling points were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the number of slugs and percentage damage, with the numbers in between calculated by polynomial interpolation. Damage assessments were carried out on 20 leaves in a 50 cm radius of each refuge trap.

Table 4.4. Pearson's Correlation Coefficient (r) for damage and slug counts on different assessment dates in five fields in Shropshire, UK. Slug counts (from refuge traps) and damage assessments were carried out on a 10 by 10 grid with a 10 metre interval between points. Significant correlations are highlighted.

Field	Date	Pearson's Correlation Coefficient (r)	t	degrees of freedom	p value
Adeney (Middle)	08/12/2015	-0.03	-0.26	98.00	0.80
Adeney (Middle)	22/12/2015	0.26	2.66	98.00	0.01
Adeney (Middle)	05/01/2016	0.32	3.34	98.00	0.00
Adeney (Middle)	18/01/2016	0.33	3.44	98.00	0.00
Adeney (Middle)	29/01/2016	0.43	4.68	98.00	<0.0001
Adeney (Middle)	12/02/2016	0.24	2.47	98.00	0.02
Adeney (Middle)	26/04/2016	0.24	2.45	98.00	0.02
Lynn (Badjics)	08/12/2015	0.39	2.44	34.00	0.02
Lynn (Badjics)	18/12/2015	0.52	5.95	98.00	<0.0001
Lynn (Badjics)	06/01/2016	0.23	2.38	98.00	0.02
Lynn (Badjics)	20/01/2016	0.30	3.09	98.00	0.00
Lynn (Badjics)	03/02/2016	0.40	4.36	98.00	<0.0001
Lynn (Badjics)	18/02/2016	0.18	1.77	98.00	0.08
Lynn (Badjics)	25/05/2016	0.16	1.56	98.00	0.12
Lynn (Stoney Lawn)	07/12/2015	-0.33	-1.54	20.00	0.14
Lynn (Stoney Lawn)	18/12/2015	-0.26	-1.37	26.00	0.18
Lynn (Stoney Lawn)	06/01/2016	-0.04	-0.43	98.00	0.67
Lynn (Stoney Lawn)	21/01/2016	-0.14	-1.37	98.00	0.17
Lynn (Stoney Lawn)	02/02/2016	-0.26	-2.71	98.00	0.01
Lynn (Stoney Lawn)	18/02/2016	-0.13	-1.27	98.00	0.21
Lynn (Stoney Lawn)	15/03/2016	0.04	0.39	98.00	0.70
Lynn (Stoney Lawn)	24/05/2016	0.09	0.85	98.00	0.40
Uppington (1)	07/12/2015	0.18	1.80	98.00	0.01
Uppington (1)	17/12/2015	0.52	5.95	98.00	<0.0001
Uppington (1)	04/01/2016	0.04	0.39	98.00	0.70
Uppington (1)	19/01/2016	0.25	2.50	98.00	0.01
Uppington (1)	01/02/2016	0.16	1.55	98.00	0.12
Uppington (1)	16/02/2016	0.04	0.39	98.00	0.69
Uppington (1)	29/04/2016	-0.10	-1.01	98.00	0.31
Uppington (1)	23/05/2016	0.05	0.44	98.00	0.66

4.4. Discussion

Deroceras reticulatum causes damage to a wide range of agricultural and horticultural crops, leading to significant economic losses (Nicholls, 2014; Twining *et al*., 2009). Slug damage to winter wheat is the result of seed hollowing or direct feeding on the leaves (Glen *et al.,* 1990b; South, 1992). The refuge traps used in this study are a measure of slug activity on the soil surface, which in turn is affected by both the size of the local population and a range of environmental conditions (which can affect the proportion of the slugs that are above or below the soil surface; Young *et al.,* 1991; Choi *et al.,* 2004). As such, damage assessments were focused on leaf feeding which is more directly related to surface activity.

Deroceras reticulatum is known to display two peaks of reproductive activity in arable fields, in the spring and autumn (Port & Port, 1986). In this study slug activity during autumn was reflected in refuge trap catches in December at all five study sites, with higher levels of activity recorded later in the field season at four of the sites. The discontinuous distribution of slugs in arable fields reported by Bohan *et al*. (2000a) and Archard *et al*. (2004) resulted in discrete patches of higher slug numbers interspersed within areas of lower slug densities being readily detected in all the fields investigated in this study. During periods of sub-optimal physical conditions, a significant proportion of the population retreats to a protected environment below the soil surface (South, 1992), where they cannot be detected using refuge traps. Consequently, as has been shown at other field sites and in other years, the patches of high slug population densities were not recorded at every sampling visit, but critically were located in the same areas of the field when they were detected, suggesting that slugs either move vertically between the upper soil horizon and the soil surface, or return to the same locations in the field when conditions are favourable. The first hypothesis "Crop patches which contain higher slug numbers can be identified in winter wheat fields using the standard trapping grid described in Chapter 2", was confirmed by this work. Analytical approaches investigating the relationship between crop damage and patches of higher slug numbers, however, must take account of the periodic appearance and disappearance of slug patches when assessed using surface refuge traps.

As was concluded in Chapter 3, the temporal stability of slug patch location, support the proposal that the quantity of slug pellets (or other control products) used in agricultural fields may be reduced by targeting application of control measures to areas of high slug densities within fields, provided a cost-effective method of defining patch location soon after or before sowing of the crop can be developed. The concept of targeting of the patches of higher slug densities during pesticide application relies on the development of an equivalent (cost-effective) detection method. The number of traps required to detect patch location across a whole field would render the use of the current labour intensive refuge traps commercially uneconomic, so alternative approaches are required (Forbes *et al.*, 2018; Petrovskaya *et al.*, 2018). In addition, the variation in numbers of slugs recorded between assessments presents a problem for farmers using refuge traps as a decision-making tool for pellet application (Glen *et al.,* 2003; Rae *et al.,* 2005). This issue may be further exacerbated by the finding that patches were more difficult to detect using refuge traps in fields with low slug densities.

The standard sampling grid developed in this work programme (Chapter 2) was used to investigate the relationship between percentage feeding damage on plants and the slug catches recorded in refuge traps at each grid point. Results were variable, with both positive and some negative correlations recorded. Where statistically significant correlations occurred between the damage assessments and slug counts they were weak, suggesting that visible slug damage may not be a reliable indicator of the location of patches of higher slug densities, even in winter wheat fields with higher slug populations. The second hypothesis, “Plant damage caused by slug feeding within 50cm of refuge traps set in the standard trapping grid is consistently and significantly related to the number of slugs caught in those traps and supports the use of plant damage as a predictor of slug patch location in commercial practice”, was not upheld by the work. This contradicts the experience of many farmers and consultants, possibly because larger slug patches causing damage over a large area of crop may be accurately identified visually, whereas small patches (which can represent a significant proportion of slug damage in a field) are less readily recognised.

The weak correlations between leaf feeding damage and refuge trap catches in winter wheat fields sampled in this study suggest that crop damage could not be used as a reliable indicator of slug population level or slug patch location. Significant reduction in winter wheat crop yields can be caused by slugs before a crop emerges as a result of feeding damage to the germ of the seed, which results in seed hollowing that prevents germination, thus reducing plant density (Glen *et al.,* 1993). The similarly weak correlation between post emergence plant density and slug numbers reported from North America (Muller-Warrant *et al*., 2014) support the conclusion that plant damage may not be a satisfactory assessment method for targeted pesticide applications. In addition, slug feeding often results in emerging seedlings being severed above the seed or at ground level, again affecting plant density (South, 1992). Such early feeding activity in patches with high numbers of slugs can result in lower plant densities in discrete areas of a crop. Where similar slug densities occur, this potentially results in greater proportional damage in such patches when compared to surrounding areas where pre-emergence feeding has not occurred, reducing the accuracy by which crop damage assessment reflects slug population size. In addition, lower plant densities at the seedling growth stages may result in increased dispersion of slugs to other areas in search of food (Hamilton and Wellington, 1981), further lowering the strength of correlations between plant density and slug numbers in later assessments. As wheat plants grow, the characteristic leaf shredding caused by slug feeding occurs. Percentage leaf area affected by shredding has been used to assess damage at this stage, but the ability of the plant to rapidly produce new leaves when actively growing (AHDB, 2018) limits its value as an indicator of slug activity.

The ability to predict slug numbers and patch location using plant damage will also be affected by other factors relating to the environment in arable fields. There is evidence that the distribution of slugs can be altered by cultivations and the distribution pattern observed during the rest of the season may require a short time to stabilise (Glen *et al.,* 2006a). Winter planted crop growth is not linear during the growing season, and can, for example, depend in part on temperatures and rainfall. These variations in crop growth rate (AHDB, 2018), combined with changes in slug population size (Port and Port, 1986; Willis *et al.,* 2008) and different rates of feeding (Wareing and Bailey, 1986) are all dependant on environmental conditions which may result in the variable leaf damage and weaker correlations with slug numbers. In laboratory experiments, soil texture, seed depth and consolidation of the soil have all been shown to affect the damage caused by slugs (Stephenson, 1975). Subsequent field experiments have shown that slug biomass, the depth of sowing and the percentage of fine soil accounted for over 94 % of the variation in crop damage observed (Glen *et al.,* 1989). There is also evidence that small slugs cause proportionally more damage to seed than larger slugs (Glen *et al.,* 2006b), which further increases the complexity of the relationship between the number of slugs present and the damage observed. Glen *et al.* (2006b) found larger slugs consumed more of each seed but smaller slugs would eat only the embryo before moving on to the next seed, once the embryo is damaged the seed is no longer viable making total seed mass consumed irrelevant to the damage observed.

In summary, the discrete and stable areas within commercial winter wheat fields in which higher slug numbers occur (demonstrated in Chapter 3) supports the proposal that reduced use of molluscicides can be achieved through targeting the treatments at such areas alone. Commercially viable approaches to defining the location of these patches remain to be established, this study indicates that crop damage assessments have only limited potential. The relationship between the number of slugs present and the damaged observed is complex and this study indicates that crop damage assessments have only limited potential. In addition, as assessment of crop emergence would occur too late to protect against seed hollowing, and subsequent leaf shredding has proven to be an inaccurate method of forecasting areas of higher slug densities, therefore neither method can be used to locate and target treatments at the areas of the field at risk. A more effective approach may involve the characterisation of key factors of the physical environment that collectively lead to the formation of localised patches of high slug numbers, and development of cost effective approaches to their use in the field offers an alternative focus.

4.5. Conclusion

The relationship between slug numbers and plant damage is complex. Observed damage is affected by a range of factors; small slugs cause proportionally more damage than larger slugs, plant growth rates vary at different times in the season, environmental factors affect slug reproduction and activity, soil texture, seed depth and cultivations will all affect the damage observed. This study has confirmed that the variability caused by such factors result in damage assessment being an unreliable indicator of slug patch location and an unsuitable candidate for use in commercial practice.

Chapter 5. Relating location of slug patches to soil characteristics

Previous chapters have demonstrated the potential for a more sustainable approach to the application of molluscicides for slug management, based on targeting applications at patches of higher numbers of slugs that are found in arable fields. A requirement for an alternative, commercially viable method of locating those patches was highlighted, as the use of refuge traps was unlikely to offer a cost-effective approach. Chapter 4 demonstrated that assessment of visible plant damage resulting from slug feeding did not define the location of patches with sufficient accuracy to support targeted application of molluscicides. This chapter investigates potential soil characteristics which may influence the location of slug patches, and which might offer an alternative approach.

5.1 Introduction

5.1.1. Slug distributions and soil characteristics

Carrick (1942) first suggested that edaphic factors, such as pH, soil moisture and organic matter, might influence the location of areas of higher slug numbers in arable fields. Few field studies investigating the relationship between slugs and soil characteristics have been conducted subsequently, with the majority of research being carried out under laboratory conditions and focusing on individual soil characteristics (moisture and temperature, Getz, 1959; pH, Wäreborn, 1970; temperature, Wareing and Bailey, 1985; organic matter, Speiser, 1999). The emerging trend from the literature suggests that pH, soil moisture and factors affecting seed bed condition are the key to understanding the distribution of slugs in arable fields.

South (1965) considered the discontinuous distribution of *D. reticulatum* in relation to several environmental factors in a grassland field, including distance from the headland, organic matter content of the soil, moisture and the stone coverage (as a percentage of the soil surface), but none of these factors were found to be significantly correlated with the distribution of slugs. More recently, the distribution of 17 species of terrestrial gastropods was related to a combination of soil characteristics in 10 km by 10 km grid squares in Iberia, using three samples from 124 grid squares taken in each year of the three-year study (Ondina *et al.*, 2004). Three groupings of slugs were identified, the first showing a preference for acidic soil with a high proportion of coarse sand (>56.4 %), the second (including *D. reticulatum*) were associated with wetter, less acidic soil with high proportions of silt and clay and a third group which showed no preference. More specifically, *D. reticulatum* were found to occur in higher numbers in soils with high pH (5.6-8.5) and calcium levels (5.3-26.0 %), an intermediate level of moisture (36.8-41.6 %) and gravel fraction (8.3-14.0 %) and a low coarse sand fraction (14.7-24.2 %), low level aeration (22.3-27.1 %) and aluminium content (0.1-0.6 %) (Ondina *et al.,* 2004). Further work is required to investigate the influence of a range of selected factors, both individually and in combination on the distribution of *D. reticulatum* in arable fields in the UK.

5.1.1.1 Organic matter

Decomposing plant material not only provides a food source for slugs (Carrick, 1942) but it also affects other soil properties such as water holding capacity, soil structure and pH. Increasing the amount of organic matter in soils can increase the water holding capacity, resulting in moisture being retained for longer during dry periods (Franzluebbers, 2002). It has been estimated that for every 1 % increase in organic matter, there is a 3.6 % increase in the volume of water held at field capacity (Hudson, 1994). Slugs are dependent on their environment for water as they are unable to regulate their own body moisture (South, 1992). In soils with high clay content increasing organic matter content can also improve water infiltration through the soil (Boekel, 1963; Hillel, 2008). This reduces the incidence of waterlogging which is known to be detrimental to slug survival (Carrick, 1942).

Organic matter also improves structure and stability in soils and increases coherence of soil particles leading to soil aggregation, which increases the size of pores between aggregates therefore decreasing the bulk density of the soil (Keller and Håkansson, 2010). The increase in soil aggregation and therefore number and size of pores will also create habitat for soil dwelling organisms including refuges for slugs (Franzluebbers, 2002). A 1 % increase in organic matter leads to a decrease in bulk density of 0.14 g/cm^3 (Bauer, 1974) (see section 5.1.1.4.). As organic matter content varies widely within and between agricultural fields, depending on factors such as cultivation methods, crop rotations and soil texture (Franzluebbers, 2002), its effect on several soil properties which may impact slugs suggests that it could be an important factor in determining slug abundance.

5.1.1.2. pH

The literature reports variable results regarding the effect of pH on slug abundance. Early research investigating the relationship between molluscs (snails) and pH, suggested that a higher number of species occurred in soils over pH 6 (Atkins and Lebour, 1923) and a similar preference for neutral and alkaline soils might be expected in slugs. Although not visible externally (as with snails) slugs have a reduced calcareous shell under the mantle, an outer layer of calcium carbonate surrounding their eggs and granules of the compound are found in their slime (South, 1992), all of which require a source calcium carbonate (which is found in more alkaline soils). Boycott (1934) collated the results of a range of studies to investigate the relationship between soil pH and slug abundance of several slug species (not including *D. reticulatum*) and found no relationship between soil pH and slug distribution. This study arrived at different conclusions to Atkins and Lebour (1923), who investigated the distribution of snails in relation to pH, suggesting that slugs may not show the same preference for calcareous soils as snails. Atkins and Lebour (1923) also suggested that there may be a difference between the habitat preference of snails with thick shells and those with thinner shells.

Investigation of the relationship between pH and slug abundance in 41 potato fields in Scotland found no correlation between soil pH, the density of slugs and the level of damage to the crop, with the highest slug densities, including *D. reticulatum*, occurring in soils with pH 5.4-6.9 with the lowest slug numbers in pH 4.8-7.0 (Carrick, 1942). Conversely, a study by Ondina *et al.* (2004) demonstrated that *D. reticulatum* preferred soils with high pH (>5.6) and calcium levels (>5.33 %). The range of pH levels studied by Ondina *et al.* (2004) was wider, 3.6 to 8.1, than that in the earlier study of potato fields (4.8 – 7.2). Thus, pH may have a role in determining slug abundance in soils with a lower pH but within the range typically found in arable fields in the UK (5.5 to 7.5; Skinner and Todd, 1998)) it may not be a factor restricting the location of slug patches in most cases.

5.1.1.3. Soil texture

Soil texture varies according to the proportion of sand (large particles), silt (medium particles) and clay (small particles). Sand particles (0.02 – 2 mm) are rounded, meaning they do not pack closely together, leaving large pores between them leading to high water infiltration rates and poor water retention in soils with a high sand content. Clay particles are much smaller (<0.002 mm) and flaky; the particles can pack tightly together resulting in smaller pore sizes between clay particles than between sand particles increasing the volume of water the soil is able to retain (Gupta and Larson, 1979). Silt particles are of medium size (0.002<0.02 mm) and display intermediate properties to both sand and clay particles (Table 5.1).

Soil properties depend on the proportion of each size fraction of particles. Soils with a high proportion of clay have a more stable structure than sandy soils, and due to the swelling and shrinking of clay particles in wet and dry conditions (Hillel, 2008), these soils have a tendency to form clods and cracks when they dry out. The clods and cracks in the soil provide essential refuges for slugs (South, 1992). The majority of cereals grown in the UK are on clayey or loamy soils, with potatoes preferentially being grown on loamy or sandy loam soils (Chase, 1981). Soil texture is widely considered to be an important factor in determining crop damage from slugs in arable fields, damage records from the 1957 and 1958 growing seasons in East Anglia showed a high correlation between crop losses in winter wheat and the soil type, the majority of damage occurring on clay loam soil, with no reports of damage on sandy soil (Gould, 1961). Ondina *et al.* (2004) further confirmed this finding whilst investigating population distributions in Iberia, with *D. reticulatum* showing a preference for soils with high silt and clay proportions. In recent literature produced by AHDB Cereals and Oilseeds (2018), soil type is identified as a risk factor for slugs, with soils with high clay and silt proportions being considered more prone to high slug numbers.

Table 5.1. Properties of the different size fractions of soil, sand (0.02-2 mm), silt (0.002-0.02 mm) and clay (<0.002 mm). (Rice, 2002; Hillel, 2008).

Property	Sand	Silt	Clay
Water holding capacity	Low	Medium to high	High
Aeration when moist	Good	Medium	Medium to poor
Water conductivity	High	Slow to medium	Slow to very slow
Soil organic matter level	Low	Medium to high	High to medium
Rate of organic matter decomposition	High	Medium	Slow
Compactability	Low	Medium	High
Shrink-swell potential	Very low	Low	Moderate to very high
Resistance to pH change	Low	Medium	High

5.1.1.4. Bulk density

Bulk density is a measure of the weight of soil in a given volume, which will vary according to soil type, compaction and as previously indicated, organic matter (Franzluebbers, 2002; Kalev and Toor, 2018). Soils with high clay content form aggregates and so will typically have a lower bulk density (Nimmo, 2004; Chaudhari *et al.,* 2013). The gaps between aggregates can provide important refuges for slugs (South, 1992; Shirley *et al.*, 2001). Compacted soils will also have a higher bulk density, as particles are forced closer together and the number and size of pores is reduced (Gupta *et al.,* 1989; Van den Akker and Soane, 2005). Another factor associated with compaction and therefore high bulk densities is low water infiltration through the soil (Gupta *et al.,* 1989; Kalev and Toor, 2018), which can result in soils becoming waterlogged thus uninhabitable for slugs.

Glen *et al.* (1989) investigated the effect of seed bed conditions (e.g. bulk density) on slug numbers and crop damage in a clay loam field sown with winter wheat. Based on behavioural observations it had previously been suggested that soils with a high bulk density will inhibit the ability of slugs to move through the soil profile, reducing the availability of refuges (Stephenson, 1975). The bulk density of most agricultural soils in the UK lies between 0.76 and 1.82 gcm^3 (Ball *et al.,* 2000). For example, Fullen (1985) found bulk densities in east Shropshire between 0.89 and 1.71 gcm^3. A bulk density value of more than 1.6 gcm^3 is considered to reflect compacted soil (Kozlowski and Pallardy, 1997). The results of the study by Glen *et al.* (1989) did not show a clear relationship between the bulk density (range 1.09 to 1.24 gcm^3, below the level indicating compacted soil) and the amount of damage in plots with different seed bed cultivations. The authors suggest that this may be due to slugs being killed by the additional cultivations, which is supported by other findings in the literature (Kennedy *et al.,* 2013).

5.1.1.5. Particle density

Particle density is the measure of the weight of individual soil particles in a given volume of soil, with the average value for agricultural soils being reported as 2.65 gcm^3 (Kalev and Toor, 2018). The particle density of a soil can be used in conjunction with bulk density to calculate soil porosity. Porosity is a measure of the proportion of soil in a given volume taken up by pores, the higher the soil porosity the more space there is in the soil (Hillel, 2008). High soil porosity allows water to infiltrate the soil at a faster rate (Hillel, 2008), indicating that the soil is not compacted and may contain cracks that slugs can use as refuges.

5.1.1.6. Infiltration

Infiltration is a measure of how quickly water moves through the soil, which is also affected by soil texture, bulk density and organic matter content (Bauer, 1974; Dexter, 2004). Soil moisture content is an important factor in determining the abundance and distribution of slugs, and is related to infiltration (Wareing and Bailey, 1985; Choi *et al.,* 2006). Where the soil is too dry (e.g. in areas of very high infiltration) slugs can be susceptible to desiccation, whereas if the soil is water-logged (infiltration rates are very low) then they are unable to use cracks in the soil as refuges and are susceptible to drowning if there is standing water on the soil surface (South, 1992).

5.1.1.7. Moisture

The soil characteristics discussed above, are factors which will change relatively slowly with time, and can remain stable for relatively long periods (months or years). In comparison, moisture and temperature can change rapidly over short periods of time. Despite this, the impact of these characteristics on slug activity is well documented. Although soil moisture and temperature influence day to day visible surface activity, prolonged periods of low temperature or low or high rainfall, can also affect population size.

Slugs cannot regulate moisture levels within their own bodies or in eggs, and with a water content of approximately 85 % rely on there being sufficient moisture in their environment to prevent desiccation. Risks to adult slugs associated with too much water in the environment (e.g. waterlogged soils) are reported in section 5.1.1.6 and in addition include inhibition of movement across the soil surface as they require a substrate on which to lay a mucus trail, restricting their ability to find refuges (Denny, 1981; South, 1992), and will prevent the development of eggs (Willis *et al.,* 2008). Carrick (1942) reported a close relationship between soil moisture, *D. reticulatum* population size and plant damage with the largest slug populations detected in soils with an average moisture content of 25 %, whereas very low slug numbers and little damage were detected in a mean moisture content of 17 %. Laboratory experiments using moisture gradients, detected two thirds of the slugs were located in a band of soil at 64 % saturation (Carrick, 1942). In another study, Ondina *et al* (2004) found that *D. reticulatum* have a preference for high soil moisture (>41.6 %), when investigating the distribution of gastropods in Iberia. Although slugs have displayed preferences for specific ranges of soil moisture content the different methods used in published research for reporting soil moisture (a saturation percentage cannot be readily compared to a soil moisture content percentage; Brouwer *et al.,* 1985) has made definition of an accurate range difficult.

Laboratory experiments have shown that slug eggs desiccate under dry conditions, with no development recorded in eggs laid in soil at 25 % saturation. Optimum moisture levels for development and hatching were reported to be between 50 and 75 % saturation (Carrick, 1942). There is evidence that slugs can manipulate the number and size of eggs they lay, producing a higher total number and higher proportion of viable eggs under optimum conditions to minimise the loss to desiccation (Willis *et al.,* 2008). They also modify their behaviour in response to ambient environmental conditions, when soil moisture is lower laying eggs further below the soil surface, where moisture levels are more stable (Carrick, 1942). Soil moisture is directly affected by rainfall but also by other soil properties such as bulk density, soil texture and organic matter which vary across fields indicating that interactions between various soil characteristics may affect slug population distributions.

5.1.1.8. Temperature

Daily temperature variation is recognised as an important factor in the surface activity of *D. reticulatum* (Carrick, 1942; Choi *et al.,* 2004). Carrick (1942) found that the optimum temperature range was 10 – 20°C, with very little activity at 0°C or 25°C. Choi *et al.* (2004) reported an optimum temperature for activity of 15°C, with the upper and lower limits being 20°C and 3°C. The optimum temperatures for feeding (14°C, Wareing and Bailey, 1985), maximum growth rate (18°C, South, 1982) and optimal egg laying conditions (18°C, Willis *et al.,* 2008) each occur within these upper and lower limits for activity. Long term periods of very hot or very cold weather can impact not only activity but the abundance of slugs as feeding, lifespan and reproduction are reduced (South, 1982; Wareing and Bailey, 1985; Willis *et al.,* 2008). In combination these studies suggest that the optimum temperature range for *D. reticulatum* activity is between 10 and 20°C, although slug activity has been recorded outside this range (Mellanby; 1961).

5.1.2. Objectives and hypotheses

The majority of the research discussed has been carried out under laboratory conditions or investigated the distribution of slugs over a very large area, necessitating broad generalisations about habitat preference. Discontinuous distribution of slug populations on a smaller (commercial field) scale in relation to soil characteristics has received less attention, but it is known that characteristics can vary significantly between defined locations. The aim of the study reported in this chapter is to investigate and consider candidate characteristics of the physical environment which may contribute to determining where patches of higher slug densities form in arable fields. The conclusions may form a basis from which a method of predicting where such patches will be located (as part of a sustainable pest management approach) in future studies, but the aim of this chapter is solely to identify an initial list of possible candidate characteristics for further research.

Objectives

- To conduct laboratory experiments to investigate the responses of adult *D. reticulatum* to different temperatures, substrate moisture, soil moisture, pH, organic matter or temperature and draw conclusions on their potential for use as a predictor of slug patch location in the field.
- To map selected characteristics of the physical environment, and slug densities in arable fields where slug patches have previously been identified, on the same standard 10 x 10, 10 m interval sampling grid.
- To compare the variability of soil characteristics across the sampling grid with the areas of higher and lower slug densities.
- To draw conclusions on which of the physical characteristics (if any) warrant further investigation as predictors (individually or in combination) of the location of patches of higher slug densities in arable fields.

Hypotheses

- When offered a choice of a range of soil moistures in laboratory experiments conducted using stepped gradients, adult slugs will display clear (statistically significant) preferences for defined moisture conditions.
- Repeating similar experiments using stepped gradients, to test adult slug preferences for a range of conditions of temperature, or pH, or the level of organic matter, specific conditions will be favoured in each case.
- Selected soil characteristics (soil organic matter content, pH, soil texture, bulk density, particle density, porosity, infiltration rate, soil moisture) will vary across standard sampling grids established at all experimental field sites, and a consistent (statistically significant) difference between the level of the individual soil

characteristics measured in areas containing patches of high slug density and areas with low slug densities will occur.

5.2 Materials and Methods

5.2.1. Laboratory experiments - Soil characteristic gradients

Eighty adult slugs (*D. reticulatum*) were collected from two field sites (Harper Adams University (52°46’01.26’N 002°34’50.14’W) and Wigan (53° 30′ 22.66″ N 002° 42′ 25.54″ W)) and returned to the laboratory for a 48 hour acclimatisation period in a Fitotron (Sanyo SGC097.CFX.F - Fitotron Temperature/Humidity Test Chamber, Weiss Technik UK Ltd, UK; 14 hours light at 15°C: 10 hours dark at 10°C, 60% humidity). As slugs are active at night, experimental assessments would need to be made during the scotophase. To facilitate this all individuals were acclimated to a light dark cycle in which scotophase commenced at 08:00.

Individual slugs were maintained in a rearing enclosure comprising a 250 ml circular plastic container (11.5 cm diameter; 4.2 cm high) with eight 1 mm diameter holes drilled through the lid. The base of each enclosure was covered with a 4 cm^2 disc of damp paper towel (2 ply blue centre feed roll, Cater4you, UK), moistened with 1 ml of distilled water, which was replaced daily. Slugs were fed ad-libitum on 1 cm thick slices of carrot which were replaced daily. Stepped soil gradients (investigating soil moisture, pH, organic matter or temperature; Table 5.2) were established. Each gradient consisted of 4 compartments (15 cm x 22 cm x 3.3 cm) made from foil trays (D-272-33, Cater For You, UK), connected by making 2.5 cm cuts in the corners of one of the long edges and folding them to create a join between adjacent compartments (Figure 5.1). A 2 cm band of Vaseline and salt mixture (ratio 4:3) was applied to the edge (but not the connecting platform) of each compartment in order to contain the slugs within the experimental arena.

Figure 5.1. Stepped soil gradients used to investigate slug preference for soil moisture, pH, organic matter (pictured) or temperature were established. Four compartments (15 cm x 22 cm x 3.3 cm) with a 2 cm band of Vaseline and salt mixture (ratio 4:3) was applied to the outer edges in order to contain the slugs within the experimental arena. Each compartment contained 330 cm^3 of air dried soil and 1 cm slices of carrot were placed in the centre of each compartment to ensure slugs had access to food at all points along the gradient.

Each individual tray was weighed before 330 cm^3 of air dried (for a minimum of 72 hours at 35°C) and sieved (through 2 mm mesh) soil (collected from 52°46’01.26’N 002°34’50.14’W) was added to each tray, providing a 1 cm deep layer, with its surface level with the joint between compartments. The compartments were then reweighed to calculate the exact weight of the soil contained. Distilled water was added to all compartments to elevate the moisture content to field capacity (16 %), apart from those used in the moisture gradient experiment (in which water was added at varying rates to create the gradient described in Table 5.2). Field capacity of the soil was calculated using the pressure membrane technique (Richards and Weaver, 1944). A total weight of each assembled compartment (including foil tray, soil, Vaseline/salt and water) was recorded for each compartment, to allow for any water lost through evaporation to be replaced at daily intervals.

Food was offered *ad-libitum* and consisted of a singular circular disc (approximate diameter 2 cm; depth 0.5 cm) of carrot, placed in the centre of each compartment. These discs were of sufficient size to ensure a constant food supply was always available in each compartment throughout the experiment. Experiments for each soil characteristic were run simultaneously and slugs were randomly assigned to an experimental gradient (soil moisture, temperature, pH or organic matter). In each replicate, 20 replicates per treatment group for moisture (low), pH, organic matter and temperature, a single pre-weighed slug was released into a compartment determined using a random number generator. A further eight slugs were collected for the moisture (high) experiment, the number of replicates in this experiment was reduced due to time constraints. Experiments were carried out in a Fitotron under the same conditions as those used during the acclimatisation period and commenced simultaneously 1 hour after the start of the scotophase. The position of each slug within each experimental arena was recorded 3 times per day at 0900, 1300 and 1700 for a five-day period.

Table 5.2. Levels of five soil characteristics tested in a stepped gradient to investigate substrate preferences of adult *Deroceras reticulatum.* Moisture = % field capacity; organic matter = % by weight.

Characteristic gradient	Compartment 1	Compartment 2	Compartment 3	Compartment 4
Moisture (low)	50 %	75 %	100 %	125 %
Moisture (high)	125 %	200 %	290 %	370 %
pH	5.86	6.26	6.51	6.97
Organic matter	0 %	3 %	6 %	9 %
Temperature	4°C	5°C	14°C	25°C

5.2.1.1. Organic matter

Air dried and sieved soil (1.5 kg) was heated in a furnace (AAF11/18, Carbolite Gero, UK) for 4 hours at 550℃ to remove organic matter (Ministry of Agriculture, 1986), before being placed in the compartments of the stepped gradient. A gradient was created by adding known percentages of compost (97 % organic matter content, Godwin's multi-purpose compost, E. J. Godwin (Peat Industries) Ltd). To calculate the organic matter content of the compost it was air dried and 10 g was added to a pre-weighed crucible. The weight of compost was recorded to nearest 0.1 µg (Precisa 262SMA-FR, Precisa Ltd, UK) before being placed in a 105°C oven (LCO/42H/DIG, Genlab, UK) for 24 hours and reweighed. The sample was then put into an ashing furnace (AAF11/18, Carbolite Gero, UK) for 4 hours at 450°C, then allowed to cool before being reweighed. The following equation was used to calculate the organic matter content.

$$\text{Organic matter \%} = \frac{(\text{dry weight} - \text{final weight}) \times 100}{\text{dry weight}}$$

(Ministry of Agriculture, 1986)

No compost was added to the soil in compartment 1 and increasing rates of compost were added to compartments 2, 3 and 4 to create a gradient (Table 5.2).

5.2.1.2. pH

The pH of the soil collected from the field was determined. Ten grams of air dried and sieved soil was placed into a 100 ml beaker 50 ml water was added before the cap was secured on the beaker and placed on an orbital shaker (HS 501 digital, IKA, Germany) at 240 RPM for 15 minutes. The pH meter used (3510 pH meter, Jenway, UK) was recalibrated after every 50 samples, using pH 4.0 (Buffer Colour Coded Solution pH 4.00 (Phthalate) Red, Fisher Scientific, UK) and pH 7.0 buffer solutions (Buffer Colour Coded Solution pH7.00 (Phosphate) Yellow, Fisher Scientific, UK). The electrode was placed in test solutions until the pH reading became stable, and was then rinsed with distilled water between samples (Ministry of Agriculture, 1986). Using pre-weighed volumes of soil and water, either citric acid or calcium carbonate was added in known quantities and the pH of the soil retested until the target pH was achieved. This was confirmed by adding the known quantity of either citric acid or calcium carbonate to 10 soil samples, for each pH level and retesting the soil pH. Using this data the amount of citric acid or calcium carbonate required to alter the pH of the soil in each compartment of the pH arena to create the pH gradient detailed in Table 5.2 was calculated.

5.2.1.3. Moisture

The field capacity of the soil was calculated using the pressure membrane technique. Air dried and sieved soil was soaked overnight in distilled water, 10 rubber rings (5.4 cm diameter, 1 cm high) were placed on a porous plate and then filled with saturated soil. The samples were placed in the pressure plate apparatus (5 bar pressure plate extractor, Soil Moisture Equipment Corporation, USA). A pressure differential of 1.3 atmospheres was applied for 6 hours. The soil within each ring was then weighed and placed into a crucible, the samples were then oven dried at 105°C until a constant weight was achieved. The samples were then reweighed and the soil moisture content of the samples after the pressure differential had been applied were calculated (Richards and Weaver, 1944). Soil moisture at field capacity was calculated as 16 %. The amount of distilled water to be added to each compartment to create the gradient (initially 50 – 125 %) was calculated as a percentage relative to field capacity (Table 5.2). A second moisture gradient from 125 to 370 % was established following the results of the initial moisture gradient experiment in order to include soil with standing surface water (370 %).

5.2.1.4. Temperature

In order to create a temperature gradient, the experimental arenas were constructed in a cold room (SCS Group, UK) set at 4℃ and 14h light: 10h dark cycle. Heat lamps (MvPower AC 220V 150W Ceramic Emitter Heater Pet Reptile Heat Lamp Bulb Black, Shenzhenshi Musen Shiyefazhanyouxiangongsi, China) and Reptile Vivarium Clamp Lamps (White 150W, Aquapet, UK) were placed at one end of the gradient and data loggers (DS1921G-F5 thermochron ibutton, Homechip, UK) set at 5 cm intervals on the soil surface along the gradient to monitor the temperature throughout the experiment.

5.2.2. Field study

Soil samples (approximately 250 g) were collected from three fields (Oadby, Leicestershire; South Kyme (1), Lincolnshire and Wigan, Lancashire) at the end of the 2016-17 growing season and three fields (Adeney (Middle), Shropshire; Uppington (2), Shropshire; Wigan, Lancashire) at the end of the 2017-18 growing season from each point on a standard grid (described in Chapter 2). The fields were selected because detailed, cropping season long assessments of slug numbers would be available from refuge trapping conducted using the same grids to investigate the presence and spatial stability of patches of higher slug numbers (reported in Chapter 3). Samples were taken from each grid point and returned to the laboratory for analysis of organic matter, pH and particle density along with a separate sample for bulk density. Only a subset of samples were analysed for soil texture due to limited resources. In addition, at each grid point infiltration rates were measured at the time of soil sample collection, and soil moisture was recorded on each visit to the field for slug assessments.

On return to the laboratory soil samples were air dried for a minimum of 36 hours at 35°C, ground using a pestle and mortar and passed through a 2 mm sieve prior to analysis.

5.2.2.1. Organic matter and pH

Organic matter content and pH of each soil sample was determined using the methods described in sections 5.2.1.1 and 5.2.1.2. respectively (Ministry of Agriculture, 1986).

5.2.2.2. Soil texture

Air dried and sieved soil (10 g) was placed into a 600 ml laboratory beaker before 20 ml hydrogen peroxide was added and the soil left to soak overnight for a minimum of 15 hours. An additional 10 ml hydrogen peroxide was then added and the beaker placed on a hot plate (SD 500 digital hotplate, Stuart Equipment, UK) set at 90°C for one hour, being stirred at 10-minute intervals and the volume maintained at 25 ml by adding distilled water as required, the solution was then boiled for 2 minutes to complete the breakdown of organic matter before being allowed to cool to the laboratory ambient temperature. The solution was poured into a beaker, ensuring all soil from the beaker and the rod were included, before 10 ml of a dispersing agent (35 g sodium hexametaphosphate and 7 g sodium carbonate in 1 L distilled water) was added and the solution placed on the orbital shaker for 10 minutes. In a pre-weighed crucible, 10 ml of a dispersing agent was oven dried overnight to determine the residual weight. After shaking, all the contents from the beaker were poured into a 500 ml measuring cylinder through a 63 µm sieve. The contents of the sieve were transferred into a pre-weighed crucible (sample a) and oven-dried (60°C). The contents of the measuring cylinder were made up to 500 ml and mixed thoroughly. A 25 ml sample was taken from 90 mm depth and transferred to a pre-weighed crucible (sample b) and oven-dried (60°C). After the solution had been allowed to settle for 7.5 hours, a second 25 ml sample from 90 mm depth was taken (sample c) and transferred to a pre-weighed crucible and oven-dried (60°C). The samples were reweighed at 24-hour intervals until they reached a constant, once a constant weight was reached the weights for sand (sample a), silt (weight sample b minus sample c) and clay (sample c) were recorded (Ministry of Agriculture, 1986; Kettler *et al.,* 2001).

Sand % = weight sample a / total weight sample a + sample b*20 * 100 %

Silt % = weight sample b minus sample c – residue weight *20/ total weight sample a + sample b*20 *100 %

Clay % = weight sample c – residue weight * 20 / total weight sample a + sample b*20 *100 %

5.2.2.3. Particle density

Oven dried and sieved soil (40 g) was placed in a pre-weighed 100 ml flask and the weight recorded before 50 ml of water were added. The mixture was allowed to stand for 5 minutes before the total volume was recorded. The total volume of soil solids and particle density calculated using the equation below.

$$\text{Particle density} = \frac{\text{oven dry weight of soil (g)}}{\text{Volume of soil (ml)}}$$

(Tan, 2005)

5.2.2.4. Bulk density

Within 10 cm of each field grid point a metal soil corer (7.5 cm diameter x 7 cm height) was fully inserted into the ground. The soil sample contained within the core was removed, and returned to the laboratory in a plastic bag, where it was transferred to a paper bag and dried for 72 h (or until a constant weight was recorded in successive assessments) at 105°C in an oven. The volume of the soil was calculated using the volume of the cylinder ($\pi r^2 h$) and bulk density was calculated using the equation below.

$$\text{Bulk density} = \frac{\text{dry weight of soil (g)}}{\text{volume of soil (ml)}}$$

(Wood, 2006)

5.2.2.5. Soil porosity

Following analysis of the soil particle density and bulk density soil porosity was calculated, using the following equation

$$\text{Soil porosity} = \frac{\text{particle density} - \text{bulk density}}{\text{Particle density}} \times 100\ \%$$

(Tan, 2005)

5.2.2.6. Infiltration rate – simplified falling head method

When the soil was close to field capacity, a metal corer (15.3 cm diameter x 14.5 cm high) was inserted 5 cm into the ground and soil moisture inside the cylinder was measured using a soil moisture probe (Field Scout TDR, Spectrum Technologies Inc., USA). Water (500 ml) was added to the cylinder and the time for this water to drain from the container was recorded using a stopwatch. The soil moisture inside the cylinder was re-measured and the rate of infiltration was measured using the following equation.

$$K_{fs} = \frac{(\Delta^{\theta})}{(1-\Delta^{\theta})\ t_a}\left[\frac{D}{(\Delta^{\theta})} - \frac{\left[D + \frac{1}{\alpha^*}\right]}{(1-\Delta^{\theta})}\ \ln\left[1 + \frac{(1-\Delta^{\theta})D}{(\Delta^{\theta})\left[D + \frac{1}{\alpha^*}\right]}\right]\right]$$

Where Δ^{θ} = difference between field-saturated water content and the initial water content, α^* = constant, D = Volume of water / cross-sectional area of the infiltrating surface, t = time

(Bagarello *et al.*, 2004)

5.2.2.7. Soil moisture

Soil moisture was recorded at 5 cm depth at each grid point on each sampling visit to the field using a soil moisture probe (Field Scout TDR, Spectrum Technologies Inc., USA).

5.2.3. Statistical analysis

All statistical analyses were conducted using R 3.3.3. (R core Team, 2015). All residuals were tested for normality and equal variance.

5.2.3.1. Laboratory experiments

The analysis of the four gradient experiments was carried out using a generalized linear mixed effect model (GLMER). Non-significant terms were removed from the model to reach a minimum adequate model.

5.2.3.2. Field study

Maps of slug counts created using the interp and filled.contour functions in R. The number of slugs in between traps was calculated by polynomial interpolation. The presence of hotspots was determined using the ScanLRTS function in R. The areas of higher slug densities were identified using the analysis carried out in Chapter 3. A Student's t test was carried out to analyse the results of each soil characteristic within areas of higher slug density compared to areas of lower slug density. Where the assumptions of normality and equal variance were not met a non-parametric Wilcoxon Mann-Whitney test was carried out.

5.3. Results

5.3.1. Soil characteristic gradients

In experiments investigating slug responses to characteristics of the physical environment, the number of slugs recorded in different compartments of the stepped gradients at the end of the 5-day exposure varied. Statistically significant differences in slug numbers between compartments were found in experiments investigating organic matter content, low moisture, high moisture and temperature gradients, but no significant difference was observed between the numbers of slugs in each compartment of the pH gradient (Table 5.3).

Table 5.3. Proportion of individual slugs recorded at the end of the 5-day experimental period in each of the four compartments of the stepped gradients used to investigate the responses of slugs to organic matter content of soil, or soil pH, moisture or temperature. Figures followed by different letters were significantly different ($p < 0.05$). Each soil characteristic were investigated separately and conditions in each compartment (C1-C4) in the five experiments were; Exp A: Organic matter content – C1= 0%, C2=3%, C3=6%, C4=9%; Exp B: pH – C1=5.86, C2=6.26, C3=6.51, C4=6.97; Exp 3: Moisture (low) – C1=50% of field capacity, C2=75%, C3=100%, C4=125%; Exp 4: Moisture (high) - C1=125%, C2=200%, C3= 290%, C4=370%; Exp 5:Temperature – C1=4°C, C2=5°C, C3=14°C, C4=25°C.

	Compartment 1	Compartment 2	Compartment 3	Compartment 4
Organic matter (n=20)	0.70 ^{a}	0.10 ^{b}	0.10 ^{b}	0.10 ^{b}
pH (n=20)	0.15 ^{a}	0.23 ^{a}	0.31 ^{a}	0.31 ^{a}
Moisture - low (n=20)	0.10 ^{a}	0.10 ^{a}	0.10 ^{a}	0.70 ^{b}
Moisture – high (n=8)	0.37 ^{a}	0.37 ^{a}	0.18 ^{a}	0.08 ^{b}
Temperature (n=12; lower due to mortalities)	0.00 ^{a}	0.46 ^{b}	0.46 ^{b}	0.08 ^{a}

5.3.1.1. Organic matter

The number of slugs recorded in the section of the gradient with 0 % organic matter at the end of the experiment was significantly higher than in all other compartments ($z=3.28$, d.f.=54,3, $p=0.001$; Table 5.3; Figure 5.2). Differences in slug distribution between compartments started to emerge during day 2 of the experiment and increased in magnitude thereafter (Figure 5.2).

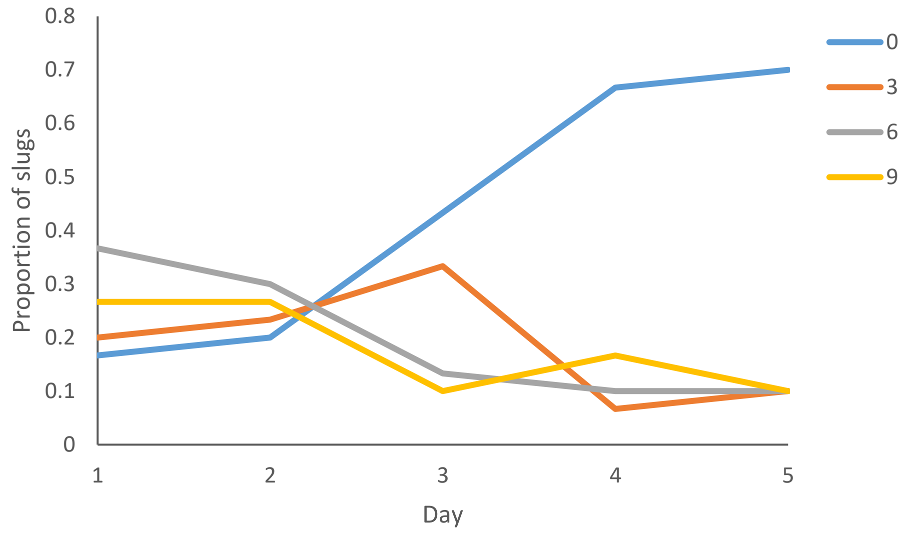


Figure 5.2. Proportion of slugs observed in each compartment of the stepped organic matter gradient at the end of each scotophase period during the five-day experimental period. The four compartments contained soil with 0, 3, 6 or 9 % organic matter content,

5.3.1.2. pH

No significant differences were detected between the number of slugs observed in the different compartments of the pH gradient at the end of the experiment (when compared with the compartment with soil pH 5.86; the compartment with soil 6.26 - $z=-0.73$, d.f.=3,84, $p=0.47$; soil pH - 6.51 $z=1.77$, d.f.=3,84, $p=0.078$; soil pH - 6.97 $z=0.89$, d.f.=3,84, $p=0.37$; Table 5.3; Figure 5.3).

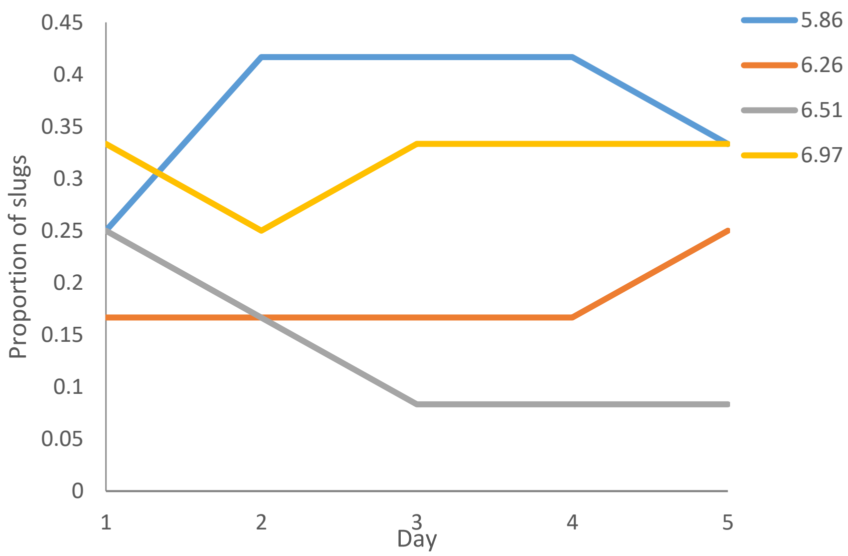


Figure 5.3. Proportion of slugs observed in each section of the stepped pH gradient at the end of each scotophase period during the five-day experimental period. The four compartments contained soil with a pH of 5.86, 6.26, 6.51 or 6.97.

5.3.1.3. Moisture

In the experiment in which the lower range of soil moisture was investigated (50-125 % field capacity), a significantly higher number of slugs were recorded in the gradient compartment offering the highest moisture content (125 %; $z=4.93$, d.f.=3,72, $p<0.001$; Table 5.3; Figure 5.4(A)). Differences between the compartments with different soil moisture were apparent from early in the experiment and was maintained thereafter (Figure 5.4(A)). When the experiment was repeated using a gradient with soil moisture ranging from 125 % to 370 % of field capacity, significantly fewer slugs were recorded in the compartment with 370 % field capacity ($z=2.99$, d.f.=3,58, $p=0.003$; Table 5.3; Figure 5.4(B)). Differences between compartments containing the two higher soil moistures, and those with lower moisture levels appeared to start emerging during day 2 of the experiment and were largely maintained thereafter (Figure 5.4(B)).

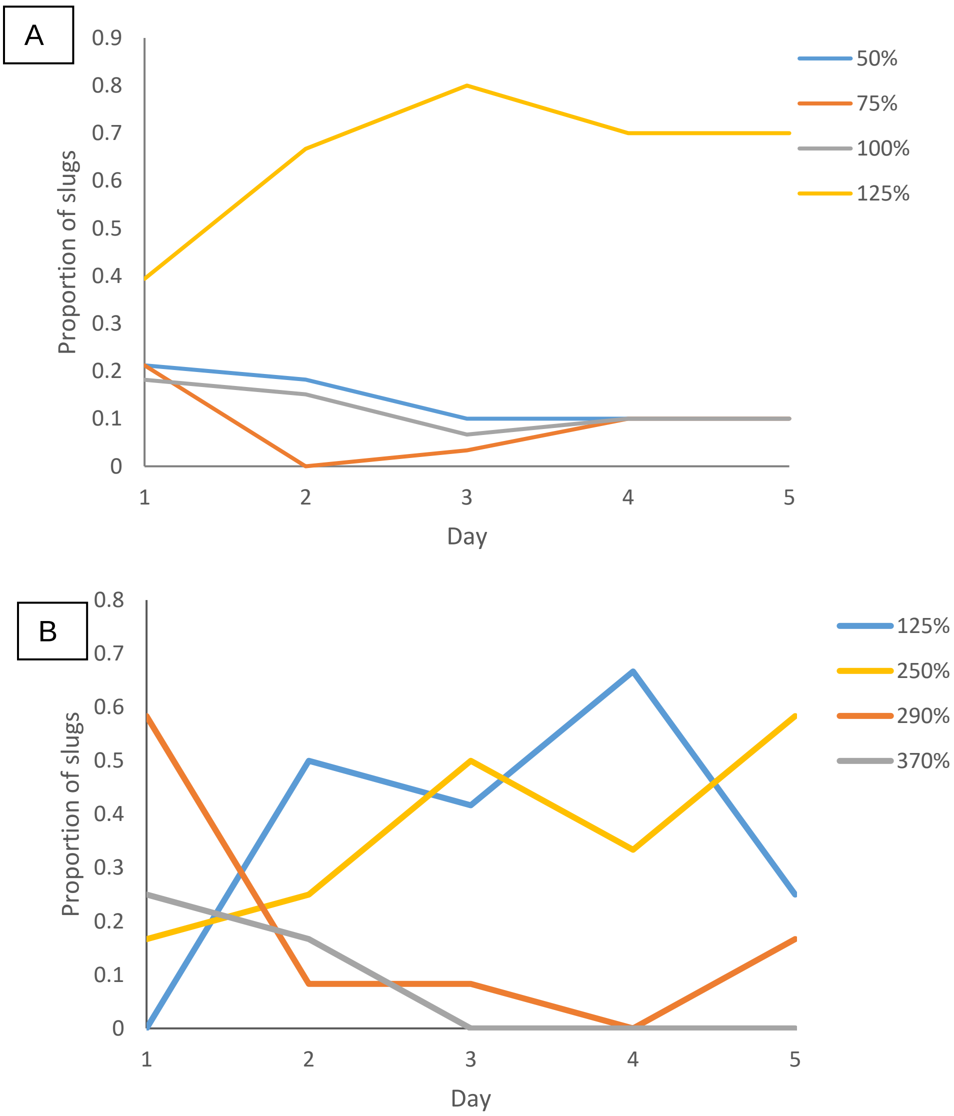


Figure 5.4. Proportion of slugs observed, at the end of each scotophase period during the five-day experimental period, in two stepped moisture gradients containing soils with moisture content (A) 50 to 125 % field capacity and (B) 125 to 370 % field capacity.

5.3.1.4. Temperature

A significantly higher number of slugs were recorded in the 5 and 14°C compartments of the stepped gradient used to investigate responses to temperature than in the more extreme temperatures offered (when compared with the compartment with the lowest temperature, 4°C; 5°C z=2.87, d.f.=3,12, p=0.004; 14°C z=2.49, d.f.=3,12, p=0.013; 24°C z=0.64, d.f.=3,12, p=0.64; Table 5.3; Figure 5.5). In addition, 3 slug mortalities were recorded in the compartment with the lowest temperature (4°C; 2 on day 1 and 1 on day 2) and 5 mortalities in the highest temperature section of the gradient (24°C; 2 on day 1, 2 on day 2 and 1 on day 3). No mortalities were recorded in the 5 and 14°C compartments. Differences between the numbers of slugs in different compartments emerged from day 2 of the experiment and were maintained thereafter until the final assessment (Figure 5.5).

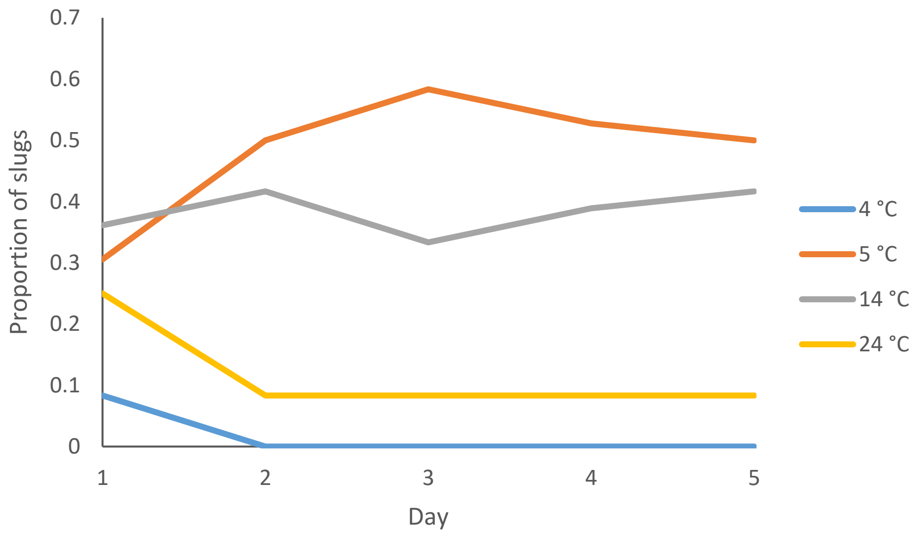


Figure 5.5. Proportion of slugs observed in each section of the temperature gradient at the end of each scotophase period during the five-day experimental period. The four compartments contained soil maintained at 4, 5, 14 or 24°C.

5.3.1.5 Selection of candidate soil characteristics for field testing

The results of the laboratory experiments suggest a range of candidate soil characteristics for further investigation in field experiments evaluating factors that affect the spatial location or temporal stability of patches of higher slug density in arable fields.

Soil moisture has long been associated with slug activity (see section 5.1.1.7) but varies rapidly with weather conditions (e.g. temperature, wind, insolation etc.) making correlation with slug numbers caught in refuge traps difficult. Its recognised importance, however, necessitates its inclusion in the planned field study, but a range of other factors that influence soil moisture levels in the field will also be considered. These include infiltration (section 5.1.1.6), and factors that can affect both water infiltration/retention or the ability of slugs to penetrate into the upper horizons of the soil such as soil texture (section 5.1.1.3), bulk density (section 5.1.1.4) and particle density (section 5.1.1.5).

Temperature was strongly associated with slug activity in the laboratory experiments but is also a factor that changes rapidly making field study difficult, and will not be included in this work programme.

The pH of soil and has also been related to slug activity in published studies (section 5.1.1.2) with variable outcomes. The laboratory experiments conducted under the current programme did not replicate published findings, but due to the importance of pH to soil organisms, the factor will be further investigated in the planned field experiment. Similarly, laboratory results relating to the organic matter content of soil also failed to replicate published findings, but the known importance of organic matter both directly to slugs and via its effect on other soil characteristics such as soil structure and water infiltration (section 5.1.1.1.; neither of the latter were tested by the laboratory experiment) warrants inclusion of the factor in the planned field investigation.

5.3.2. Soil characteristics and slug distribution in arable fields

Inter-field variation between the soil characteristics assessed was detected in the six arable fields investigated (organic matter, $F=834.3$, d.f.=5,594, $p<0.001$; pH, $F=120.5$, d.f.=5,594, $p<0.001$; bulk density, $F=298.2$, d.f.=5,594, $p<0.001$; particle density (due to limited resources a subset of samples were analysed for particle density), $F=3.6$, d.f.=5,48, $p<0.008$; porosity, $F=329.0$, d.f.=5,594, $p<0.001$; infiltration rate (due to unsuitable conditions infiltration rates were not recorded in the 2017-18 season in Wigan (2017-18) or at some grid points in Adeney (Middle) or Uppington (2), $F=29.3$, d.f.=4,453, $p<0.001$; moisture, $F=508.5$, 5,594, $p<0.001$; Table 5.4). In four of the six fields significant differences were detected in the level of at least one of the soil characteristics between traps located in the patches of high or low slug densities, as defined in Chapter 3 (Table 5.5). For example, in the 2016-17 season in Oadby bulk density ($t=-2.13$, d.f.=98, $p=0.036$) and porosity ($t=2.08$, d.f.=98, $p=0.040$) and in Wigan organic matter content ($t=2.40$, d.f.=98, $p=0.018$) and pH ($t=2.03$, d.f.=98, $p=0.045$) were found to differ significantly between patches of higher slug numbers and the spaces between these patches which contained lower slug densities. In the 2017-18 season pH in Adeney (Middle) ($t=2.51$, d.f.=98, $p=0.014$) and the maximum soil moisture content at the Wigan site ($t=2.04$, d.f.=98, $p=0.044$) were also found to vary significantly between areas in which slug patches had formed and those areas not incorporated into patches. In addition, assessments of soil texture were taken at a single field site (Adeney (Middle)) and significant differences were recorded between percentage sand ($t=-3.89$, d.f.=21, $p<0.001$), percentage silt ($t=3.41$, d.f.=21, $p=0.002$) and percentage clay ($t=3.12$, d.f.=21, $p=0.005$) recorded in soil samples from within patches of higher slug densities and the areas between these patches.

Table 5.4. Mean (± standard error) values for soil characteristics sampled in a 100 m by 100 m area of each of three arable fields in each of two cropping years, using the 10 by 10 grid with a 10 metre interval between nodes. Where letters differ, a significant difference occurs ($p<0.05$).

Year	Field	Organic matter %	pH	Bulk density gcm^{-3}	Particle density	Porosity %	Infiltration rate mm/s	Moisture %
2016-17	Oadby	8.2 ±0.05 a	5.9 ±0.03 a	1.2 ±0.01 a	2.4 ±0.04 a	50.0 ±0.4 a	6.1 ±0.6 ab	34.9 ± 0.33 a
	South Kyme	9.6 ±0.1 b	7.0 ±0.05 b	1.2 ±0.01 b	2.2 ±0.03 b	47.1 ±0.3 b	4.9 ±0.7 ab	48.9 ± 0.34 b
	Wigan	6.8 ±0.1 c	6.2 ±0.03 cd	1.3 ±0.01 c	2.4 ±0.02 a	47.8 ±0.5 c	2.6 ±0.5 a	30.5 ± 0.24 c
2017-18	Adeney (Middle)	5.1 ±0.05 d	6.1 ±0.03 c	1.3 ±0.01 c	2.3 ±0.06 ab	41.9 ±0.4 d	9.4 ±1.7 b	36.5 ± 0.34 d
	Uppington (2)	3.2 ±0.04 e	6.3 ±0.04 d	1.6 ±0.01 d	2.4 ±0.03 a	31.3 ±0.3 e	25.9 ±3.9 c	27.6 ± 0.34 e
	Wigan	4.5 ±0.1 F	6.6 ±0.02 e	1.3 ±0.01 b	2.4 ±0.04 a	44.2 ±0.4 f	*	34.8 ± 0.35 a

*no infiltration data collected as large cracks present in soil

Table 5.5. The range of soil characteristics in areas of fields assessed for patches of higher slug densities. In each field stable slug patches were detected using catches of refuge traps set at each node of a standard 10 by 10 grid, with a 10 metre interval between nodes. Soil assessments were taken at the same nodes. Figures represent mean slug count across the whole trapping grid, or for the maximum and minimum value for each soil characteristic. Values highlighted in grey indicate significant (p <0.05) differences between areas within and outside slug patches. * = no measurements taken.

Fields 2016-17	**Slug numbers**	**Organic matter %**	**pH**	**Texture-sand %**	**Texture - silt %**	**Texture - clay %**	**Bulk density gcm^{-3}**	**Particle density gcm^{-3}**	**Porosity %**	**Infiltration rate mms^{-1}**	**Mean moisture %**	**Max. moisture %**	**Min. moisture %**
Oadby	0-6	6.4-9.6	5.2-6.7				0.97-1.52	2.4	36.7-65.0	0-32	26-44	36-57	11-33
South Kyme (1)	0-6	7.1-12.4	5.8-7.9				0.95-1.33	2.2	39.5-56.8	0.1-58	39-57	46-69	20-52
Wigan	0-24	3.9-9.3	5.5-6.8				1.02-1.46	2.4	38.8-58.3	0-48	23.5-36.5	31-51	4-29
Fields 2017-18													
Adeney (Middle)	0-6	3.6-6.8	5.2-6.9	28.7-49.8	16.6-29.4	31.4-43.3	1.0-1.55	2.3	32.4-56.5	0-75	28-45	33-52	17-38
Uppington (2)	0-31	2.4-4	5.5-7.5				1.36-1.79	2.4	35.4-43.1	0-90	20-37	24-47	11-29
Wigan	0-8	2.2-7.2	6.1-7.3				1.12-1.52	2.4	36.6-53.2	*	25-42	29-50	20-37

5.3.2.1. Organic matter

The organic matter content of individual soil samples in the six fields sampled varied from 2.2 % (Wigan 2017-18) to 12.4 % (South Kyme (1) 2016-17; Table 5.5). Although laboratory experiments showed no differences in slug activity between soils with enhanced organic matter content, a significant correlation between slug numbers and soil characteristics was detected at one of the six study sites. The spatial variation in the organic matter content of the soil at the Wigan site in 2016-17 is illustrated in Figure 5.6(A). The discrete patches of higher slug densities were defined using the method described in Chapter 3, an example of the distribution at the Wigan site is shown in Figure 5.6(B). The level of organic matter in soils adjacent to refuge traps set within patches of high slug densities (7.06) was compared with and found to be significantly greater than those in areas of lower slug densities (6.55 %; t=2.4, d.f.=98, p=0.018). The Wigan site displayed the largest range of organic matter (3.9-9.3 %; Table 5.5) potentially indicating that sufficiently large spatial variation is required before the effect of the factor can be detected using the methods employed. South Kyme (1) displayed a range that was only slighter lower than at Wigan (7.1-12.4 %; Table 5.5), but with no significant correlation between slug numbers and soil organic matter. The lowest level of organic matter content in this case was higher (7.1 % in South Kyme (1) compared to 3.9 % in Wigan).

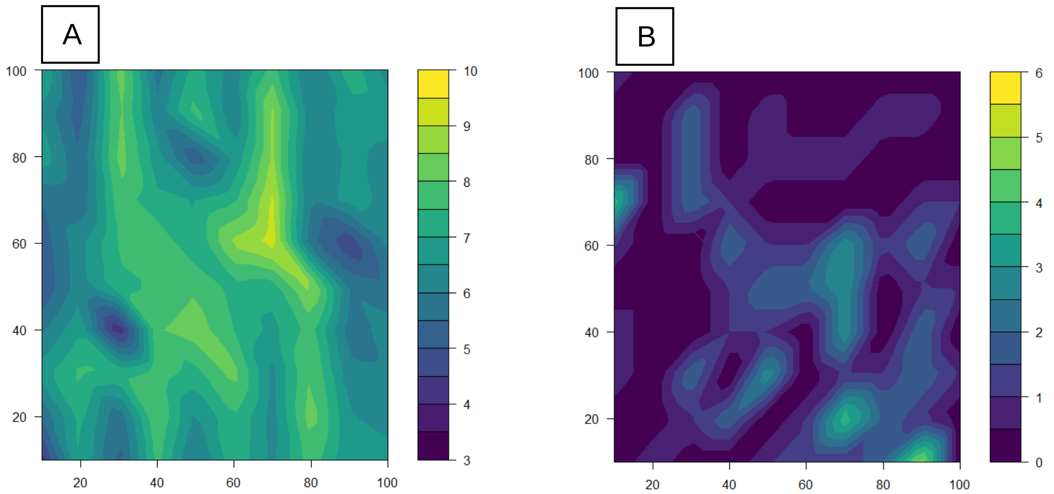


Figure 5.6. The percentage of organic matter (A) and an illustration of the distribution of slugs in Wigan (2-3-17) (B) at each point on the standard sampling grid. The x and y axes show dimensions of the sampling grid in metres. Slug distributions were determined from 10 assessments carried out between October 2016 and June 2017 using 100 refuge traps positioned at 10 metre intervals in the 10 by 10 grid. Soil samples for organic matter content were taken from the same positions. Colour scale represents the organic matter (A) and number of slugs (B), the numbers between traps was calculated by polynomial interpolation.

5.3.2.2. pH

The pH of individual soil samples in the six fields sampled varied from 5.2 (Oadby 2016-17 and Adeney (Middle) 2017-18) to 7.9 (South Kyme (1) 2016-17; Table 5.5). There was a significant difference in the pH level of the soil samples taken from areas of higher slug density when compared with those with lower slug density at two of the sites investigated (Table 5.5). The spatial variation in soil pH across the sampling grid at the Wigan site in 2016-17 is illustrated in Figure 5.7(A), an example of the slug distribution at the Wigan site is shown in Figure 5.7(B). The mean pH of soil samples taken within slug patches (6.28) was found to be significantly higher than in those samples taken from areas with lower slug densities (6.16; t=2.03, d.f.=98, p=0.045). In Adeney (Middle) in the 2017-18 comparison of the spatial variation in soil pH across the sampling grid (Figure 5.8(A)) showed that the average pH in patches containing higher slug densities (6.32) was also significantly greater than in areas with lower slug densities (6.16; t=2.51, d.f.=98, p=0.014; Figure 5.8).

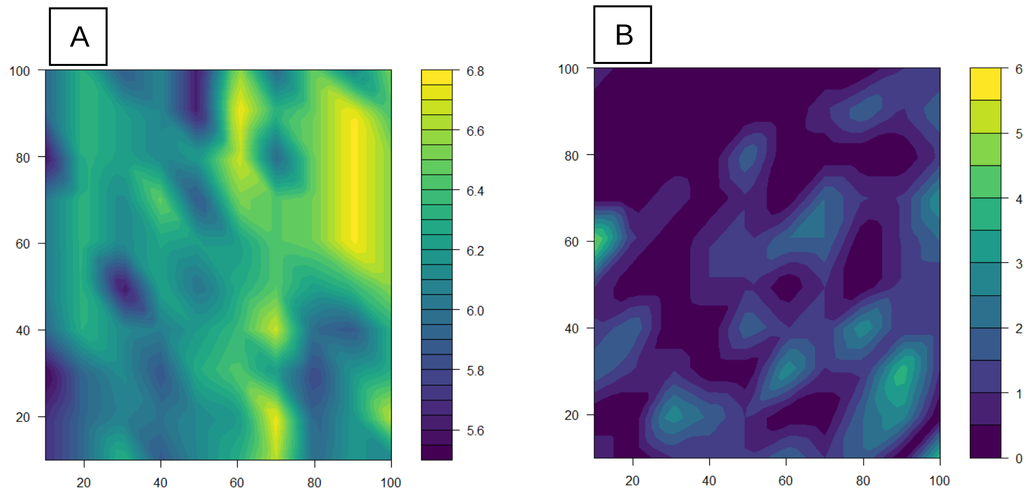


Figure 5.7. The pH of the soil (A) and an illustration of the distribution of slugs in Wigan (22-3-17) (B) at each point on the standard sampling grid. The x and y axes show dimensions of the sampling grid in metres. Slug distributions were determined from 10 assessments carried out between October 2016 and June 2017 using 100 refuge traps positioned at 10 metre intervals in the 10 by 10 grid. Soil samples for pH were taken from the same positions. Colour scale represents the pH (A) and number of slugs (B), the numbers in between traps was calculated by polynomial interpolation.

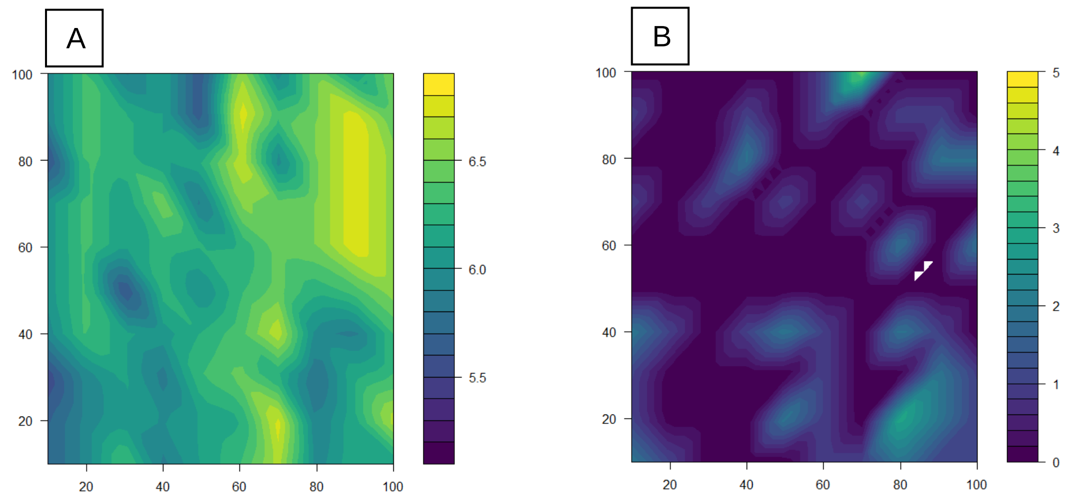


Figure 5.8. The pH of the soil (A) and an illustration of the distribution of slugs in Adeney (Middle) (20-12-17) (B) at each point on the standard sampling grid. The x and y axes show dimensions of the sampling grid in metres. Slug distributions were determined from 8 assessments carried out between September 2017 and May 2018 using 100 refuge traps positioned at 10 metre intervals in the 10 by 10 grid. Soil samples for pH were taken from the same positions. Colour scale represents the pH (A) and number of slugs (B), the numbers in between traps was calculated by polynomial interpolation.

5.3.2.3. Moisture

Soil moisture was assessed at each node of the standard sampling grid, on each assessment visit to all six field sites. The individual soil moisture measurements recorded in the fields ranged from the lowest of 4 % (Wigan, 2016-17) to the highest of 69 % moisture content (South Kyme (1) 2016-17; Table 5.5). Soil moisture measurements varied between assessments, for example, at the Wigan site the mean soil moisture content was 27.7 % on 12-4-17, 9.5 % on 10-5-17 and 30.9 % on 8-6-17. No significant differences in either the mean soil moisture (mean for each point calculated from individual measurements from each assessment) or minimum soil moisture (minimum recorded for each point on the grid on any assessment date) assessments between the areas of higher or lower slug densities were identified (Table 5.5). A significant difference was detected between the maximum moisture content of the soil in areas of higher slug density (43.1) when compared to areas of lower slug density (40.6%) in a single site, Wigan (2017-18) ($t=2.76$, d.f.=98, $p=0.010$). The spatial variation in maximum soil moisture content and an illustration of slug distribution across the sampling grid at Wigan are shown in Figure 5.9.

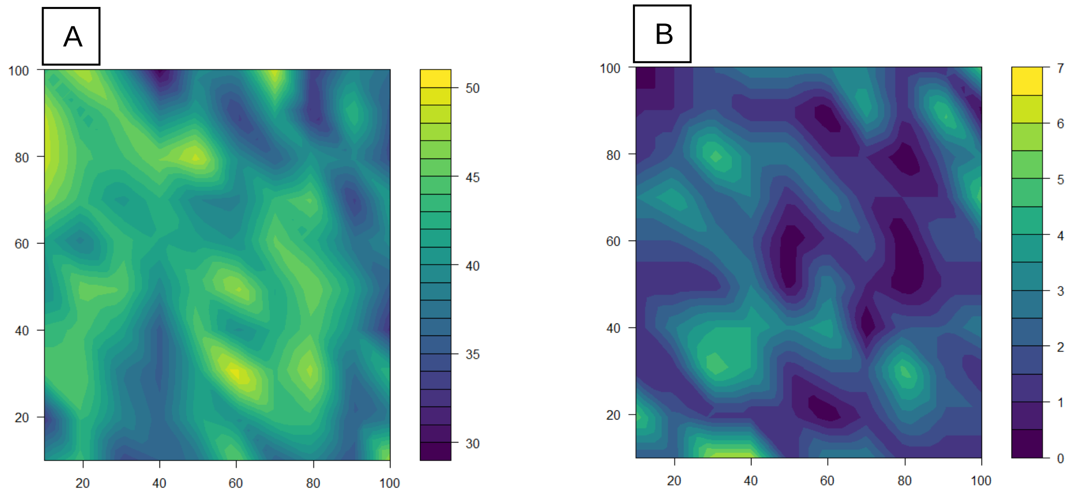


Figure 5.9. The maximum moisture content of the soil (A) and the distribution of slugs in Wigan (12-1-18) (B) at each trap location on the grid. The numbers along the x and y axis show dimensions of the sampling grid in metres. 100 refuge traps were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the maximum moisture content (A) and number of slugs (B), the numbers in between traps was calculated by polynomial interpolation.

5.3.2.4. Soil texture

Due to limited resources soil texture analysis was carried out on a subset of samples in only one field (Adeney (Middle) 2016-17). There was a significant difference in the percentage of each particle size fraction between soil taken from areas containing higher slug densities and those with lower densities, clay ($t=3.12$, d.f.=21, $p=0.005$), silt ($t=3.41$, d.f.=21, $p=0.003$) and sand ($t=-3.89$, d.f.=21, $p<0.001$) (Figure 5.10).

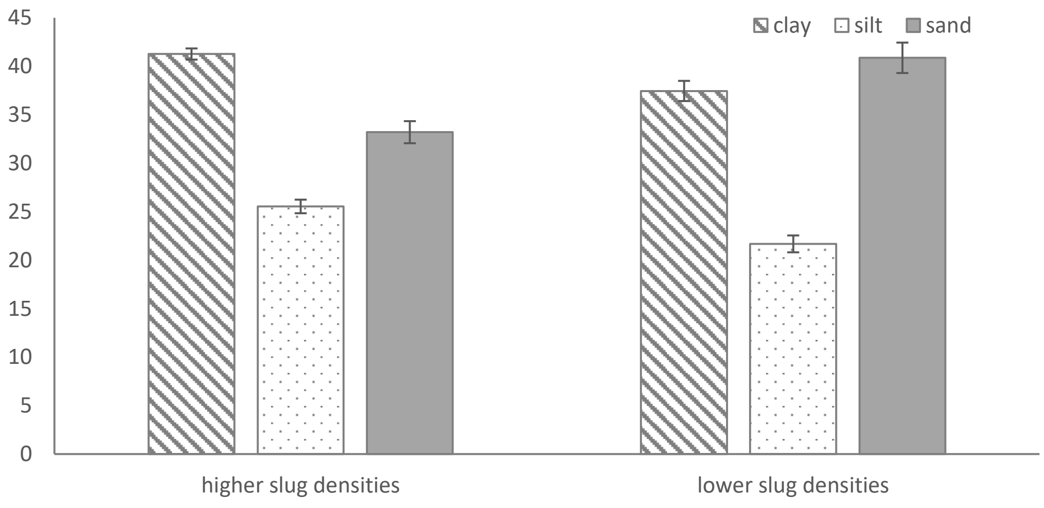


Figure 5.10. The relative proportions of sand, silt and clay in soil samples taken within patches of higher slug densities and in areas of lower densities in Adeney (Middle) (2016-17), n=23. Error bars show the standard error.

The average proportion of clay in soils taken from within the area of higher slug density was significantly higher (41.3 %) than in those from areas with lower densities (37.5 %).

Similarly, a higher proportion of silt was found in samples from within the area of higher slug density (25.5 %) compared to other areas (21.7 %). Conversely, the proportion of sand in the area of higher slug density was lower (33.2 %) compared to the area of lower slug density (40.8 %) (Figure 5.10).

5.3.2.5. Bulk density

Bulk density recorded in individual samples from all six experimental fields ranged from 0.95 gcm^{-3} (South Kyme (1) 2016-17) to 1.79 gcm^{-3} (Uppington (2) 2017-18; Table 5.5). A significant difference in the bulk density of the soil was detected between areas of higher slug density compared to areas of lower slug density in only one field. At Oadby in the 2016-17 season the average bulk density of the soil in patches of higher slug density was 1.18 gcm^{-3} compared with 1.22 gcm^{-3} in the patches with a lower density ($t=-2.13$, $d.f.=98$, $p=0.036$; Figure 5.11). The bulk density measurements at Oadby covered the largest range of those recorded in all six study sites.

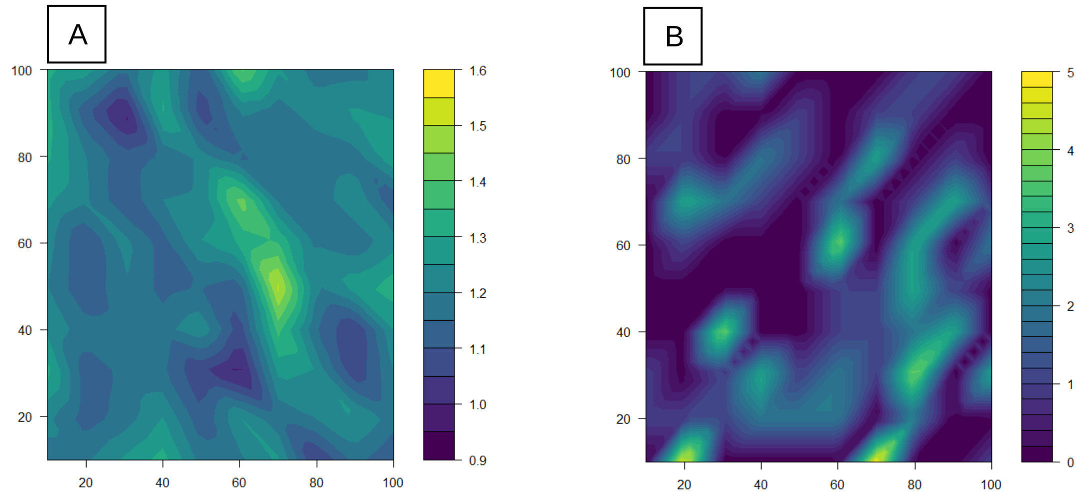


Figure 5.11. The bulk density of the soil (A) and an illustration of the distribution of slugs in Oadby (2-9-16) (B) at each point on the standard sampling grid. The x and y axes show dimensions of the sampling grid in metres. Slug distributions were determined from 8 assessments carried out between September 2016 and June 2017 using 100 refuge traps positioned at 10 metre intervals in the 10 by 10 grid. Bulk density samples were taken from the same positions. Colour scale represents the bulk density (A) and number of slugs (B), the numbers in between traps was calculated by polynomial interpolation.

5.3.2.6. Particle density

A subset of samples were analysed for particle density, little variation between individual samples was detected, for example, the mean of 26 samples taken from Wigan 2017-18 was 2.41 ±0.03 gcm^{-3}. As the initial analysis indicated that the differences between samples (±0.03 gcm^{-3}) were less than the accuracy of the technique (±0.2 gcm^{-3}) and time resources were limited it was decided not to continue with particle density measurements at individual grid points. Instead sample points were collated from nine areas of the grid and the mean particle density for the field used in the calculation for soil porosity measurements.

5.3.2.7. Soil porosity

Soil porosity is a measure of the proportion of a defined volume of soil that is taken up by pores and can be calculated from measurements of bulk density and particle density, and as such is not independent of these two factors. The soil porosity recorded in individual soil samples taken from the six fields investigated ranged from 32.4 % (Adeney (Middle) 2017-18) to 65.0 % (Oadby 2016-17). A significant difference in the porosity of the soil was detected in areas of higher slug density compared to areas of lower slug density at one site, Oadby (2016-17). At Oadby the average soil porosity in patches of higher slug density was 50.8 % compared to 49.1 % in the areas with lower slug density (t=2.08, d.f.=98, p=0.040). The spatial variation in soil porosity and an illustration of slug distribution at Oadby (2016-17) are shown in Figure 5.12.

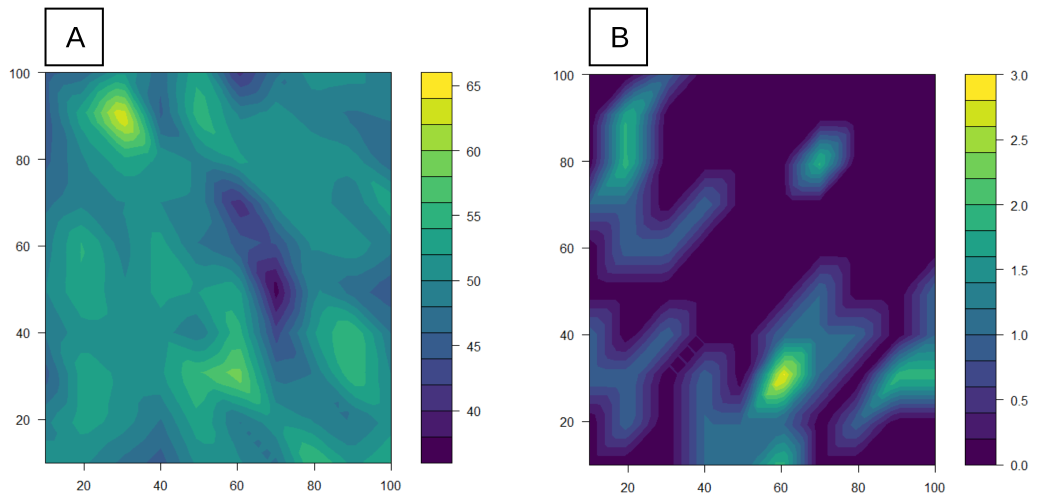


Figure 5.12. The porosity of the soil (A) and an illustration of the distribution of slugs in Oadby (2-9-16) (B) at each point on the standard sampling grid. The x and y axes show dimensions of the sampling grid in metres. Slug distributions were determined from 8 assessments carried out between September 2016 and June 2017 using 100 refuge traps positioned at 10 metre intervals in the 10 by 10 grid. Porosity for each point was calculated using bulk density and particle density measurements. Colour scale represents the soil porosity (A) and number of slugs (B), the numbers in between traps was calculated by polynomial interpolation.

5.3.2.8. Infiltration rate

Infiltration rates were assessed in five of the fields investigated and outcomes ranged from the lowest individual assessment of 0 mms^{-1} (occurring in all fields except South Kyme (1) 2016-17) to the highest rate of 90 mms^{-1} (Uppington (2) 2017-18). No significant difference in the infiltration rate was detected in areas of higher slug density compared with areas of lower slug density in any of the fields tested.

5.4 Discussion

Development of a commercially viable integrated pest management system in which control measures are targeted at patches of higher slug densities (as defined in Chapter 3), relies on a cost-effective method of identifying the location of those patches. Assessment of visible crop damage was not found to be sufficiently reliable (Chapter 4), and use of soil characteristics that affect slug behaviour and population development were proposed as an alternative approach. In order to identify candidates which may have a role in determining slug location, laboratory experiments were first carried out to identify a set of characteristics that may influence the location of slugs in the field. These candidate characteristics were then tested along with selected additional characteristics in a field study.

Decomposing plant material provides both a food source for slugs and can modify other properties of soil including water holding capacity, soil structure and pH. Increasing the amount of organic matter in soils can improve access of slugs to the upper soil horizons, providing refuges from predators or during periods of adverse environmental conditions (Franzluebbers, 2002; Keller and Håkansson, 2010). A study manipulating organic matter on forest floors found that a reduction was associated with a reduction in the population density of Collembola (Eaton *et al.,* 2004). The underlying mechanism may lie in leaf litter not only providing a food source and refuge for the Collembola but also regulating the temperature and moisture of the forest floor. Increased organic matter content can also increase the water holding capacity resulting in moisture being retained for longer during dry periods, an important feature as slugs are unable to regulate their own body moisture (South, 1992; Hudson, 1994). Slugs have been reported to show a preference for soils containing high levels of organic matter levels suggesting that variation in organic matter content across fields may influence their distribution (Carrick, 1942; South, 1992). Laboratory tests conducted in the current study using a stepped gradient, however, resulted in slugs aggregating in compartments with no added organic matter rather than those with supplemented organic matter content. This may have been due to the substrate used (a commercial multi-purpose compost) to increase organic matter content, as anaerobically digested matter has previously been found to deter slugs. Speiser (1999) reported slug repellency with 96 % of the individuals tested avoiding lettuce that was offered in association with anaerobically digested matter in a choice test. Further field tests showed significantly lower lettuce leaf loss from slug feeding in plots treated with newly digested matter compared to untreated plots or those treated with older digested matter, or with a barrier of newly digested matter around the plot (Speiser, 1999). Preliminary choice tests have been carried out which show farmyard manure does not elicit the same repellent response as the compost used in this study (Patel, 2018). Further testing is required using different substrates to confirm the slug preference for higher levels of organic matter widely reported in the literature. Therefore, although the laboratory experiments in this current study did not provide evidence supporting the inclusion of organic matter for further testing in field experiments it was selected as a candidate soil characteristic based on evidence from the published literature.

Slugs require a source of calcium carbonate as a reduced calcareous shell is present under the mantle, granules of the compound are found in their slime, and a layer of calcium occurs in eggs (South, 1992). Calcium carbonate is found in more alkaline soils, suggesting that pH may influence habitat choice, although the findings of studies investigating the effect of pH on slug and snail abundance are variable. Carrick, (1942) investigated slug populations in potato crops but found no consistent relationship between soil pH and the density of slugs. The highest slug densities (including *D. reticulatum)* recorded, occurred when soil pH was within the range 5.4-6.9, with the lowest slug numbers found in soil ranging from pH 4.8-7.0. Investigating a wider range of soil pH (4.8 – 7.2), Ondina *et al.* (2004) demonstrated that *D. reticulatum* preferred soils with a pH >5.6. Although the laboratory experiment conducted in the current study did not demonstrate a preference for specific soil pH, this may have been the result of the limited range tested (pH 5.9-7.0) which spans the levels that earlier studies suggest are preferred. Soil pH may have a role in determining slug abundance but within the range typically found in arable fields in the UK (5.5 to 7.5; Skinner and Todd, 1998) it may not be a primary determinant of the location of slug patches. Collectively, however, these findings suggest that further work is required to identify the optimum pH range for slugs, and accordingly, it was retained as a candidate soil characteristic for field investigation.

Slug responses to the laboratory moisture gradient in this current study indicated a significant preference for moist soils, a comparable result to the findings of Carrick (1942). Moisture is widely known to play a role in surface activity (South, 1992; Shirley *et al.,* 2001; Choi *et al.,* 2004), with slugs showing a preference for damp but not waterlogged soils (Young and Port, 1991; Glen and Symondson, 2003). The second moisture gradient included compartments with waterlogged soils, which were avoided by slugs, again supporting published observations. These results suggest that soil moisture may be an important characteristic influencing the location of higher slug densities but practical considerations preclude its use in a viable IPM approach. Soil moisture is affected by a range of weather (e.g. insolation, temperature, wind etc.) and environmental factors (e.g. water retention capacity of the soil, which in turn is affected by multiple soil characteristics), resulting in high temporal variability. Such variability results in soil characteristics that affect water retention being more attractive candidates for inclusion in the field experiment, including soil texture, bulk density, particle density, porosity and infiltration rate).

Studies of the effect of temperature have reported low slug activity below 5°C (South, 1992). Activity increases as temperatures exceed 10°C (Young *et al.,* 1991), with an optimum of between 13°C (in winter) and 17°C (summer; Wareing and Bailey, 1985). The temperature gradient experiment conducted in the current study in part supported these findings, with significantly fewer slugs at the end of the experiment located in the compartments with the lowest (4°C) and highest (24°C) temperatures than in those with intermediate temperature of 14°C. A similar number of slugs were found, however, in the compartments with temperatures of 5°C and 14°C. A higher rate of mortality was observed in the temperature gradient experiment when compared to the other laboratory studies. In order to create the gradient, a heat lamp was used to raise the temperature at one end of the continuum. Although moisture lost through evaporation was replaced daily, the compartments in the temperature gradient required more water to be replaced than in the organic matter, pH and moisture experiments. The highest mortality rate was recorded in the compartment with the highest temperature (24°C), potentially the result of reduced soil moisture and the lack of refuges within the compartment resulting in desiccation, rather than as a direct result of the heat. A series of experiments investigating tolerance of *D. reticulatum* to temperature extremes found slugs to be tolerant of temperatures up to 36°C. The same series of experiments investigated desiccation and found all individuals died between 2 and 12 hours after being subjected to dry conditions (Getz, 1959), time periods that are shorter than those used in this study. Although soil temperature significantly affects slug activity, as was the case for soil moisture, temperature in the upper horizons of the soil and above the soil surface is highly variable, and can vary widely in relatively short periods of time. As such it was not selected for further study in the field experiment but should remain a consideration for farmers when considering the optimum timing for slug control applications.

The results of the laboratory gradient experiment investigating soil moisture support the first hypothesis that “When offered a choice of a range of soil moistures in laboratory experiments conducted using stepped gradients, adult slugs will display clear (statistically significant) preferences for defined moisture conditions”. The second hypothesis “Repeating similar experiments using stepped gradients, to test adult slug preferences for a range of conditions of temperature, or pH, or the level of organic matter, specific conditions will be favoured in each case” was not fully supported. Although statistically significant preferences for temperature were identified, no differences between the four levels of soil pH offered to slugs were detected. In addition, although significant differences were established between slug preferences for the unenhanced soil samples when compared to those to which various amounts of organic matter had been added, no preference was found between the variable organic matter content of the enhanced soils.

5.4.1. The impact of soil characteristics under field conditions

The laboratory gradient experiments demonstrated that slugs may identify and select some soil conditions that are most favourable for their survival, and may favour areas of fields offering such conditions, forming patches with higher slug densities. The location of such patches may not, therefore, be solely reliant on the availability and quality of the food plants within the patch (provided a deficit in food availability does not occur). This supports the hypothesis that soil characteristics may indicate the location of areas of higher slug densities in arable fields.

Based on the results of the laboratory experiments and literature sources, nine soil characteristics were selected for further investigation in a field study conducted over two years in commercial winter wheat fields (section 5.3.1.5). These included soil organic matter content, pH and other physical factors that affect water retention (soil texture (% sand, % silt, % clay), bulk density, particle density, porosity, and infiltration rate. In addition, regular measurements of soil moisture were taken.

Although variable between sites and years the field study did demonstrate that variation in the soil characteristics within fields may influence the location of the areas of higher density of *D. reticulatum*. Six of the soil characteristics were found to vary significantly between patches of higher and lower slug densities. The lack of consistency between fields or years, however, suggests that a single individual factor cannot be used to reliably predict the locations that are at risk of the development of higher slug numbers. Further work is required to develop an improved understanding of the effect of each characteristic, for example, whether an optimal range or critical threshold exist which may allow more accurate definition of favourable habitat conditions.

Accordingly, several characteristics may have to be used in combination to improve the accuracy of predictions of patch location. For example, the importance of soil moisture in slug habitats is widely recognised but is influenced by a range of soil characteristics affecting water retention. Of those assessed in this experiment, soil taken from within or outside slug patches differed significantly in their sand, silt and clay content, or in organic matter, bulk density, and porosity, in at least one field. By combining assessments of these related factors, and establishing optimum ranges or thresholds for each, they might be layered to create a more accurate map of areas with favourable slug habitats within each field. Further work is required to investigate the potential of this approach but the current study has identified some candidate soil characteristics.

At South Kyme (1), the field site in which organic matter was highest, there was no significant difference between the organic matter content within and outside the areas of higher slug densities. This was despite the difference between organic matter content of the two sets of samples being the second largest recorded in any of the fields studied, possibly indicating that the importance of this factor is lower when a generally high (and adequate) soil organic matter content is found throughout the field. In the field with a similar range (Wigan (2016-17; 3.9-9.3 %), but starting at a lower organic matter content, a significant difference between the area of higher and lower slug densities was detected. Further work is required to identify whether there is a critical threshold for organic matter content in determining the location of slugs in arable fields.

Lewis and Magnuson (2000) investigated spatial patterns of species richness of aquatic snails in relation to variations in environmental conditions. They found that calcium content of the water, pH and habitat availability could all influence the species richness of a lake and the distribution within individual lakes. In this study, pH was significantly higher within areas of higher slug density in two of the three fields with the lowest pH of the fields sampled, reflecting the results of Ondina *et al* (2004) who suggested that *D. reticulatum* have a preference for soils with a higher pH (>5.60).

Results from Adeney (Middle), the only site at which soil texture was analysed, suggest that further investigation of soil texture in a greater number of fields is warranted. A significant difference was found between soil textures in areas with higher slug density when compared to those with lower slug density. The higher clay content of the soil, coupled with a higher silt and lower sand content within the area of higher slug density, links the patch location with soils displaying higher water retention. The proportion of sand, silt and clay affects the water retention properties of soil. Soils with high proportions of sand display poor water retention and are more prone to drying, whilst those with a high proportion of clay have better water retention properties. Soils with high silt content are intermediate between the two (Rice, 2002; Hillel, 2008). This is consistent with evidence from the literature that slugs show a preference for heavier soils with a higher clay content, partly because of the higher moisture retention characteristics (Gould, 1961; South, 1992; Ondina *et al.,* 2004; AHDB, 2016). Firm conclusions cannot be drawn from results from the single field site, but soil texture is identified as a candidate for future studies.

Bulk density and associated porosity are influenced by soil texture and organic matter content (Franzluebbers, 2002; Kalev and Toor, 2018), but they are also affected by compaction, caused by cultivation method and the weather conditions at the time of cultivation (Franzluebbers, 2002; Batey, 2009). As a result, bulk density cannot be directly correlated with soil texture and organic matter. Soils with higher bulk densities display lower water infiltration rates and those above 1.6 gcm^{-3} are considered to be compacted (Kozlowski and Pallardy, 1997). Uppington (2) was found to have areas of compaction, with bulk densities up to 1.79 gcm^{-3} detected. The effect of compaction may result in fewer large aggregates and cracks in the soil, resulting in fewer available refuges for slugs in those areas. A lack of refuges has been associated with increased mortality (Shirley *et al.,* 2001). Based on behavioural observations it has been suggest that even in soils that are not compacted, a higher bulk density can inhibit the ability of slugs to move through the soil profile, reducing the availability of refuges (Stephenson, 1975). A significant difference was detected between the bulk densities recorded within patches of higher slug density (1.18gcm^{-3}) and areas of lower slug density (1.22 gcm^{3}) at the Oadby site, making this another candidate for future investigation.

No significant relationship was found in any of the fields sampled, between infiltration rate and the density of slugs, perhaps in part due to the large variation in infiltration rates between adjacent traps. The simplified falling head method used in this study has been found to be a good measure of the mean infiltration rate but does result in much higher variation than both the double ring infiltrometer (Verbist *et al.,* 2010) and pressure infiltrometer techniques (Bagarello *et al.,* 2012). The alternative methods require longer recording time and more equipment (pressure infiltrometer method) reducing the number of possible readings from each of the fields sampled. In order to maximise the accuracy of the simplified falling head method for measuring the rate of infiltration, measurements should be taken shortly after rainfall when the soil moisture is close to field capacity. The 2017-18 season was particularly dry, which prevented infiltration rates being recorded in one field, due to the occurrence of cracks that allowed water to dissipate quickly or the formation of a crust on the soil surface preventing water movement down the soil profile. The variation in measurements and necessity of certain soil conditions makes infiltration a less suitable characteristic for determining the location of areas of higher slug densities in commercial crops.

Moisture and temperature are widely known to influence slug activity (Choi *et al.,* 2004), however, soil moisture was only found to be a significant factor at one site, Wigan. Although recognised as an important factor affecting the behaviour and activity of slugs in arable fields, soil moisture varies widely and on a short temporal scale in the field, making it difficult to utilise as a reliable indicator of the areas of fields that are at risk of developing patches of higher slug densities. Instead, soil characteristics that affect the retention of moisture, such as soil texture, bulk density, particle density, porosity, or infiltration rate may offer greater potential. Both temperature and surface moisture may, however, play a more crucial role in determining the optimal timing of molluscicide pellet applications (Shirley *et al.,* 2001). Surface activity of slugs is affected by weather conditions and applying controls at a time when surface activity is highest would increase the number of slugs exposed to the pellets, improving the efficacy of treatment applications.

The third hypothesis (defined in section 2.1.2.) “Selected soil characteristics (soil organic matter content, pH, soil texture, bulk density, particle density, porosity, infiltration rate, moisture) will vary across standard sampling grids established at all experimental field sites, and a consistent (statistically significant) difference between the level of the individual soil characteristics measured in areas containing patches of high slug density and areas with low slug densities will occur”, was not supported by the results of the field experiments.

When planning future work, in addition to the soil characteristics suggested by the current study, additional factors that also affect habitat quality and which were not investigated should also be considered, such as compaction and chemical composition (e.g. calcium content) (Ondina *et al.,* 2004). In addition to physical factors, biological factors may also be important in determining the spatial distribution of a population (Lewis and Magnuson, 2000). Predators of slugs such as carabid beetles are known to have a discontinuous distribution across arable fields (Bohan *et al.,* 2000b) and slug populations may be limited even in otherwise suitable environmental conditions by the presence of these predators.

5.5. Conclusion

Although no one individual soil factor was associated with slug patch location, several candidate characteristics (organic matter, bulk density, porosity, soil texture (percentage of sand, silt and clay) and pH) were identified as being significantly different between areas of higher and lower slug densities in at least one of the fields studied. These factors are known to influence soil moisture and/or the soil structure (providing access for slugs to the upper horizons of the soil). Further investigation of these factors is required in a larger number of fields to improve our understanding of each characteristic both individually and in combination. For example, the existence of optimal ranges or critical thresholds for each characteristic should be considered. Additional research into the stability of these soil characteristics over time is also required. This work should also include an investigation of the potential for improving our understanding of the role of soil (and other) factors, such as calcium content and level of predation, in determining the location of patches of higher slug densities, by investigating combinations of related factors. In order to be part of a commercially viable tool for identifying patch location, the selected characteristics would need to be sufficiently stable over time.

Chapter 6. Using Radio Frequency Identification tags to track the movement of *Deroceras reticulatum* above and below the soil surface

To mitigate for risks associated with the breakdown of slug patches an improved understanding of the behaviours which lead to patch formation and cohesion is required. Patches may result from the cumulative effect of individuals ranging continuously across whole fields, but spending longer periods in more localised areas that offer environmental conditions that are beneficial to their survival or reproduction, than in other less attractive areas. Under this scenario damage will be more widespread and reliable targeting may not be possible. Alternatively, if slugs display a more restricted range and remain in such areas for long periods of time retreating into soil during periods of adverse physical conditions, slug patches may be more reliably stable and targeting of applications of control agents is likely to be more reliable. A third potential mechanism involves temporary arrestment following the chance encounter of slugs with other individuals (or signs of the presence of other individuals) of the same species, which cumulatively may promote the formation of patches of higher densities. The known habit of slime trail following (Wareing, 1986) may, for example, contribute to the development of the discontinuous distribution of some slug species. This chapter investigates slug locomotor behaviour in arable fields to determine the mechanism driving patch formation. Current slug monitoring techniques do not allow for determining individual slug location using assessments taken in a time series over several weeks. A technique using RFID technology which allowed the movement of individual slugs to be monitored was developed.

6.1. Introduction

6.1.1. Understanding the mechanism underlying slug patch formation

Few studies have investigated the behavioural responses that influence the formation of areas of higher slug densities (patches) or their spatial or temporal stability. Difficulties associated with studying and effectively tracking *D. reticulatum* in the field have hampered investigations. A large but variable proportion of the slug population in arable fields is located beneath the soil surface, with a smaller proportion active on the soil surface (South, 1992), resulting in the number of surface-active slugs varying widely under different environmental conditions. In cold or dry weather a smaller proportion of the population will be observed on the soil surface as slugs move down the soil profile where conditions remain more constant (Choi *et al.,* 2006). Various techniques have been developed to assess populations, including surface searching, refuge traps, hand sorting of soil, soil flooding, DATs and capture-recapture approaches (South, 1992), and have been used to confirm the non-uniform distribution of *D. reticulatum*. The lack of data on slug population size and individual movement beneath the soil surface, contributes to there being no conclusive findings on the mechanisms underpinning the formation, stability or location of higher density patches (Forbes *et al.*, 2017).

6.1.2. Tracking individual slugs

Previous studies of the behaviour of *D. reticulatum* have attempted to track the movement of individuals using approaches such as freeze-marking (marks made on the mantle using hot copper wire irons; Richter, 1976), dye injected into the slug (Hogan and Steele, 1986), UV dye (Foltan and Konvicka, 2008) and radioactive isotopes (Hakvoort and Schmidt, 2002). A common problem with these methods is the requirement for the slug to be on the soil surface in order for it to be located and individually identified. In addition, the markers can be short-lived or difficult to distinguish in the field making the identification of individuals difficult. For example, the radioactive isotopes used by Hakvoort and Schmidt (2002) could only be identified for approximately 10 days after the radioactive feed source was removed. Moreover, the freeze-marks applied to the slug's mantle by Richter (1976) only lasted for up to 2 months on mature *D. reticulatum* while the injected dyes used by Hogan and Steele (1986) were difficult to detect on darker individuals making them hard to distinguish in the field.

6.1.3. Radio frequency identification technology

Radio Frequency Identification (RFID) tags have been used to track movement in a range of vertebrate and invertebrate species, including fish (Roussel *et al.,* 2000), honey bees entering and exiting hives (Henry *et al.,* 2012) and vine weevils (Pope *et al*., 2015), and the technology allows individuals to be uniquely identified. Additionally, fields tests showed RFID tags buried in the upper horizons of soil can be detected at least 20 cm below the surface. Tests were carried out in the field using RFID tags in eppendorfs, which were buried at different depths (from 10 cm up to a maximum of 30 cm) and using the HPR Plus reader (Biomark, USA) and BP Plus Portable antenna (Biomark, USA) located. The tags buried up to 20 cm were repeatedly located, those buried at 25 and 30 cm depth were only picked up by the HPR Plus reader on 60 and 40 % of occasions respectively (repeated 5 times at each depth). Whilst the technique has a high potential for tracking slug movement and behaviour, regardless of their position in the soil profile, few studies have attempted to develop its use. Grimm (1996) injected RFID tags into the foot of *Arion lusitanicus*, a much larger (<13 cm long) slug species than *D. reticulatum* (<5 cm), and demonstrated that tag insertion had no effect on survival and egg laying, although no work was done to establish the impact on either feeding or locomotor behaviour. The technique has since been employed by Ryser *et al*. (2011) to assess field survival rates of *A. lusitanicus* and *A. rufus* and by Knop *et al*. (2013) to investigate locomotor activity of *A. lusitanicus* and *A. rufus* in arable fields. In both cases, however, the method was used as in a mark-recapture technique rather than for tracking the movement of individuals. The use of RFID tags to study the behaviour of the much smaller slug species such as *D. reticulatum* has not been investigated to date. In order to be an effective method for tracking individual slugs, the method of marking must not affect key aspects of biology and behaviour, must allow for differentiation between individuals, and be sufficiently long-lasting.

6.1.4. Objectives and hypotheses

This study investigated potential behavioural mechanisms that lead to establishment and cohesion of areas of arable fields with higher numbers of slugs with the aim of drawing conclusions on the likely combination of responses that result in the appearance of stable patches.

Objectives

- To develop a method of attaching RFID tags to *D. reticulatum*, and testing the effects of the process on survival, feeding, egg laying and locomotion of individual slugs.
- To investigate the free movement of individual slugs in arable fields using RFID tracking to establish the extent of the range of individual slugs.
- To draw conclusions on the potential importance of behavioural mechanisms on the formation, cohesion and stability of slug patches.

Hypotheses

- RFID tags can be implanted within the body cavity of *D. reticulatum*, without, (following a defined recovery period) affecting survival, feeding, egg laying and locomotion of the individual.
- The free movement of individual slugs in arable fields can be tracked over extended periods of time using RFID technology.
- Unless challenged by adverse conditions, individual slugs remain within a small, defined area of arable fields, with lateral movement restricted to less than 20 m for extended periods of time.

6.2. Materials and Methods

6.2.1. Laboratory studies

Deroceras reticulatum were collected using surface refuge traps baited with approximately 75g chicken feed pellets (Young, 1990) from two field sites in Uppington (52°40’36.68’N 002°34’50.14’W) and Adeney (52°46’01.26’N 002°34’50.14’W), Shropshire, UK during the two-week period before the start of each experiment (between January 2016 and November 2017). Slugs weighing over 300 mg were returned to the laboratory and maintained individually in 250 ml circular plastic rearing containers (11.5 cm diameter; 4.2

cm high) with 1 mm diameter puncture holes in the lid. The base of each container was lined with paper towel (approximately 2 cm x 3 cm) moistened with 5 ml distilled water, which was replaced daily. Lettuce leaves (cv. Romaine) were offered *ad libitum* to each slug as food, and replaced with fresh leaves daily. Slugs were maintained in a controlled environment room under standard rearing conditions of 60 % humidity, 10:14 hour light: dark cycle, and at 15°C during the light phase and 10°C during dark, to reflect UK conditions in autumn and spring, and allowed a 48-hour acclimatisation period before being used in experiments.

6.2.2. Insertion of RFID Tag

To insert an RFID tag, each slug was removed from its rearing container and placed individually into a smaller circular lidded plastic container (28 ml, height 33 mm, top diameter 44 mm, base diameter 31 mm) with a 5 mm hole drilled through the top. CO_2 was gently released through the hole into the container using a Corkmaster CO_2 dispenser and 8 g CO_2 bulb (Sparklets, UK), for approximately 20 seconds or until the slug was fully extended. The anaesthetised slug was then removed from the pot and held between the thumb and index finger either side of the mantle with the head facing away from the technician. The needle of an MK165 implanter (Biomark, USA) was then positioned at an approximately 30° angle to the body wall (left side), level with the top of the keel, and ¾ of the way along the length of the slug from the anterior end. With the tip of the needle pointing toward anterior end, it was inserted through the body wall and when no longer visible, the tag (a chip and antenna coil encased in glass, 8 mm long and 1 mm wide) (HPT8 tag, Biomark, USA) was released before withdrawing the needle from the slug.

6.2.3. Treatments

Five treatments, with 20 slugs per treatment, were used to assess the effect of different aspects of the tagging process:

Tagged (T) + CO_2 + Glue (G) – slugs were anaesthetised using CO_2, an RFID tag inserted and glue (Loctite Precision Max, Loctite, USA), applied over the insertion site to seal the wound.

Tagged (T) + CO_2 - slugs were anaesthetised using CO_2 and an RFID tag was inserted.

CO_2+ - slugs were anaesthetised and the implanter needle was inserted through the body wall but no tag was injected.

CO_2 - slugs were anaesthetised with CO_2 only.

U - untreated control (slugs were maintained in the rearing cages without any part of the tag implanting process being applied).

6.2.4. Slug survival

Following RFID tag insertion, slugs were returned to their individual rearing containers and maintained under the conditions described above for 28 days. During this period, slug mortality, defined as a lack of response to a mechanical stimulus, coupled with a characteristic change in body form following death (body extended and shrivelled), was recorded at 24 h intervals throughout the experiment. Mortality assessments were confirmed when similar observations were recorded for three consecutive days). The experiment was replicated three times.

6.2.5. Feeding

RFID tagged slugs were maintained under the conditions described above for 28 days. To assess relative rate of food consumption between treatments, each slug was offered pre-weighed lettuce (approx. 1.5 g). After 24 h the remaining lettuce was re-weighed and consumption estimated by subtraction and replaced with fresh lettuce. The procedure was repeated throughout the 28-day experimental period.

6.2.6. Production of egg batches

The impact of implanting RFID tags on rate of reproduction was assessed by recording the number of egg batches laid at 24-hour intervals throughout the 28 days period following treatment.

6.2.7. Locomotor behaviour

Slugs were maintained in the laboratory for a 48-hour acclimatisation period following tag implantation under the conditions described above, before they were randomly allocated to one of two treatment groups. Slugs in the first group were implanted with an RFID tag and those allotted to the second treatment remained untagged (controls). All tags were inserted using the procedure described above (T + CO2; no glue was applied to the insertion site), and both tagged and untagged control slugs were then maintained under the standard rearing conditions for 14 days before being used for behavioural recordings. Lettuce was fed *ad libitum* and replaced daily throughout this period.

On days 14, 21 and 28 after insertion of the RFID tags, the slugs were released individually at the centre of a 50 cm diameter arena comprised of a circular plywood board painted with white gloss paint (Colours Pure brilliant white Gloss Wood & metal paint B&Q, UK). The recordings took place between 2 and 8 hours after the lights came on in the controlled environment rooms, with the order of slugs being randomised on each recording occasion. A video-camera (SONY HDR-CX240E Handycam, SONY, Japan) was positioned at 100 cm above the centre of the arena and focussed to record slug activity over the whole arena. Slug behaviour was continuously recorded for 60 minutes or until it had left the arena, whichever occurred first. Video recordings were uploaded into

Ethovision XT (Noldus, The Netherlands) and analysed for total distance moved and mean velocity. Distance moved was assessed using the centre point of the slug, which risked additional distance being added when the slug contracted and the size of its profile changed. To control for this the Ethovision settings were adjusted to ensure that a new point along the track was only recorded once the slug had moved more than 0.25 cm. The length of time it took for the slug to leave the arena was also recorded.

6.2.8. Locomotor behaviour of D. reticulatum in winter wheat

The behaviour of the slugs was investigated in commercial winter wheat crops in Shropshire, UK (52°46'01.26'N 002°34'50.14'W), in spring (April; 9 slugs) and autumn (November; 20 slugs) 2017. A 4 x 5 grid of refuge traps (as described in Chapter 2) was established in the study area, with 2 m between adjacent traps. Slugs were collected from these traps and the grid node at which each individual was caught recorded. After sufficient specimens had been collected, the traps were removed and each was replaced with a fibreglass flexi-cane to mark the grid nodes.

Slugs were returned to the laboratory where an RFID tag was inserted (each with a unique identifying code) into individual slugs using the technique described above (T + CO_2; i.e. without the application of glue), before being maintained under the standard rearing conditions for a 14-day recovery period. Individual slugs were then released (at sunset) back into the study grid at the node from which they were originally collected.

Movement was tracked after release by recording the location of the slugs at predetermined intervals using a HPR Plus reader (Biomark, USA) and a combination of two antennae (BP Plus Portable antenna; Racket antenna; Biomark, USA). Initially the racket antenna (which has a smaller read range (up to 10 cm) facilitating more accurate determination of location) was used to systematically search the area within a 1 m radius of the last known location of the slug. If the slug was not found the larger BP Plus Portable antenna (read range up to 20 cm) was used, allowing the area contained within ever larger concentric circles to be searched efficiently until the slug was located. In cases where the BP Plus Portable antenna was used to find the RFID tag in a wider area, a more precise location was then determined using the racket antenna. When an RFID tag was detected the identity of the slug was confirmed using the unique identifying code, its precise position was confirmed visually (if on the surface), and its position marked using a labelled peg recording the identifying code, assessment number, and the time of the observation. In addition, records of the slug presence above or below the soil surface, and its current activity, (leaf eating, linear locomotion, etc.) were made. Slugs were tracked at approximately 20-minute intervals for two hours post release in April 2017 and for 8 hours post-release in November 2017. In November 2017 slugs were also tracked on the following two nights for 8 hours. Following these initial periods of intense monitoring, slugs were tracked daily, and then at weekly intervals for a maximum of 38 days or until a period of 2 weeks had elapsed without any movement being observed.

Immediate accurate measurement of the distances travelled by *D. reticulatum* were more challenging during evening assessments. Accordingly, the distance between sequential marker pegs were measured the following morning. To avoid accumulation of errors that may accrue if measurements were made between sequential marker pegs, the location of each marker peg in relation to the original release point (marked by the flexi-cane on the grid node) were determined before the distance between sequential marker pegs was calculated. The location of each peg was also recorded using a hand-held GPS accurate to 18 mm (Leica RX1220T, Germany). On each night of tracking and on subsequent visits to the field, soil moisture was recorded (three points across the grid) using a soil moisture probe (Field Scout TDR 100, Spectrum technologies, Inc, USA) and soil and air temperature were recorded at 30-minute intervals using data loggers (iButton DS1921G-F5 thermochrons, Maxim integrated Products, USA).

6.2.9. Statistical analyses

6.2.9.1. Effect of implanting RFID tags on survival, feeding and production of egg batches

Following tests for normality and heterogeneity of the data (using the diagnostic plots in R to check residuals vs fitted values, Q-Q plots, scale-location plots and residual vs leverage plots), the effect of treatment on mortality rate, lettuce consumption and production of egg batches was investigated using repeated measures ANOVA.

6.2.9.2. Effect of implanting RFID tags on locomotor behaviour

Following tests for normality and heterogeneity of the data (using the diagnostic plots in R to check residuals vs fitted values, Q-Q plots, scale-location plots and residual vs leverage plots), the effect of treatment on mean velocity and total distance moved was investigated using ANOVA.

6.2.9.3. Locomotor behaviour of D. reticulatum in winter wheat

Maps of individual slug movement in the field were created using the ‘plot’ function in R. The mean total distance moved over the experimental period and the mean distance from the start point at the end of the trial period were calculated. Distances moved were calculated using linear interpolation of the x and y coordinates of two consecutive tracking points, the distance between each point and the total displacement (distance between the final location and the original release point) were calculated using Pythagoras’ theorem. The distances between each point were added together to give a total distance moved. Daily temperature and rainfall were correlated with the number of active slugs using Pearson’s Correlation Coefficient.

6.3. Results

6.3.1. Laboratory studies

6.3.1.1. Survival

Over the full experimental period a significantly lower survival rate of *D. reticulatum* was recorded in treatments in which RFID tags were implanted into slugs (T + CO_2 + G and T + CO_2) ($F=45.8$, d.f.=4,10, $p<0.001$; Figure 6.1). During the seven days after tag insertion, a mean of 5.8±1.7 of the 20 slugs in the treatment groups with an implanted RFID tag (T + CO_2 + G and T + CO_2) died compared with an average of 0.9±0.3 slugs in each of the treatment groups with no RFID tag inserted (CO_2+, CO_2 and U) (Figure 6.1).

During the 14 days post-insertion, mortality had risen to 8.1±1.1of the 20 slugs in groups with an RFID tag inserted (T + CO_2 + G and T + CO_2), and 1.3±0.4 of the 20 in those groups without tags (CO_2+, CO_2 and U). After day 15, slug survival was unaffected by the RFID tag insertion. Between day 15 and 28 there was no statistically significant difference in mortality recorded in different treatment groups ($F=3.4$, d.f.=4,8, $p>0.05$) irrespective of whether an RFID tag had been implanted. Mortality in both the tagged and untagged treatment groups was low from day 15 to 28, with a mean of 0.26 slugs per day dying in the treatment groups with an implanted RFID tag (T + CO_2 + G and T + CO_2) and 0.09 slugs per day in each of the treatment groups with no RFID tag inserted (CO_2+, CO_2 and U).

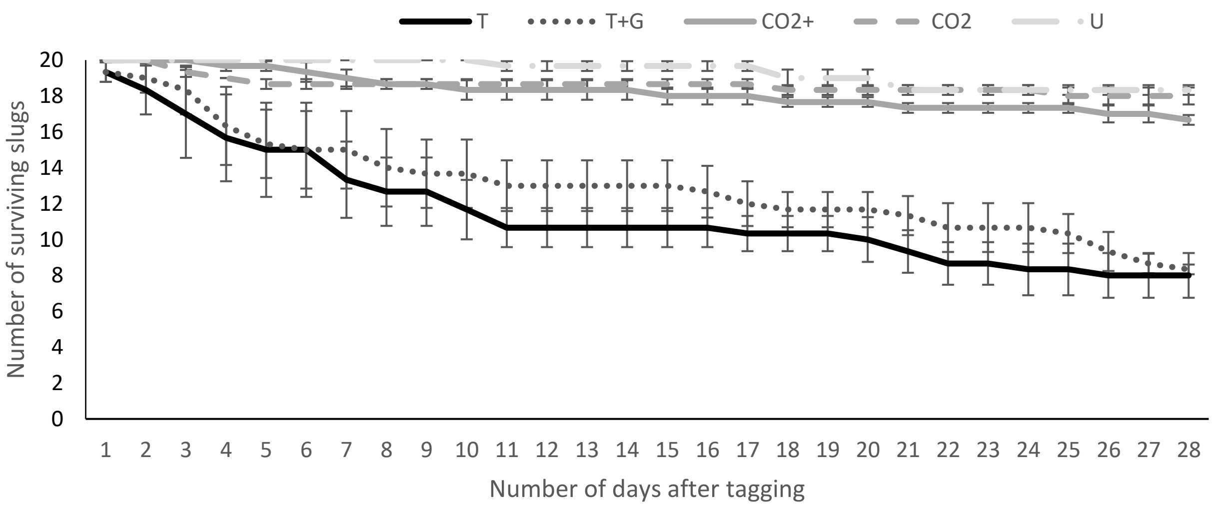


Figure 6.1. The effect of treatments on the survival of *Deroceras reticulatum* (n=100) over three 28-day periods. Mortality was recorded daily for 28 days post-tag insertion. Points show the mean for the treatment group, bars show ± SE. (T (Tag inserted) + CO_2) Slug anaesthetised using CO_2, a hole made in the body wall using the tag implanter and an RFID tag injected. (T + CO_2 + G (Glue applied to insertion site)) Slug anaesthetised using CO_2 and with an RFID tag injected as in treatment Tag inserted + CO_2, but with the resultant hole in the body sealed with the addition of a small drop of glue (Loctite Precision Max, Loctite, USA). (CO_2+) Slug anaesthetised using CO_2, a hole made in the body wall using the tag implanter but without injection of an RFID tag. (CO_2) Slug anaesthetised using CO_2 only. (U) Untreated slug.

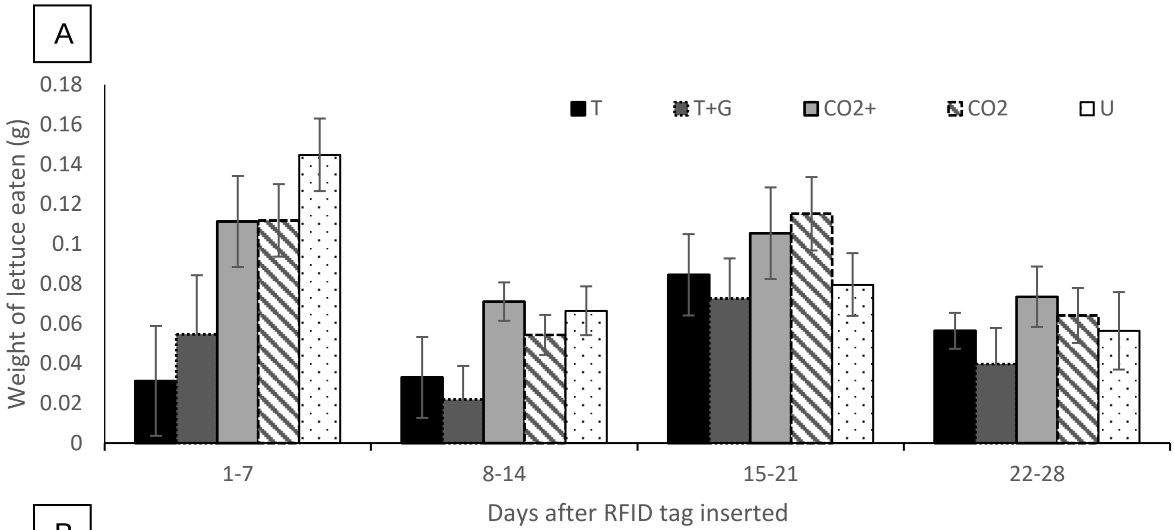
6.3.1.2. Lettuce consumption

Over the full experimental period a significantly lower daily consumption of lettuce was recorded in treatments in which RFID tags had been implanted into slugs (T + CO_2 + G and T + CO_2) ($F=10.1$, d.f.=4,1977, $p<0.001$; Figure 6.2(A)). During the 7-day period after tag insertion, slugs consumed a mean of 0.03±0.03 g per day in the T + CO_2 treatment group and 0.05±0.03 g for the T + CO_2 + G treatment group, compared to 0.14±0.02 g for the control group (U), 0.11±0.02 g for the CO_2+ treatment group and 0.11±0.02 g for the CO_2 treatment group (Figure 6.2(A)).

A significant interaction between treatment group and day was observed ($F=7.3$, d.f.=4,1977, $p<0.001$) indicating that the initial effect of treatment reduced over time. From day 15 to day 28 no significant difference in lettuce consumption was recorded between treatment groups irrespective of tagging status ($F=1.2$, d.f.=4,960, $p>0.05$), indicating a sustained and full recovery in food consumption rate by those tagged slugs that survived the procedure, occurred after an initial period of reduced intake.

6.3.1.3. Egg production

There was no statistically significant effect of treatment on the number of batches of eggs laid by slugs surviving the full 28-day experimental period over the first seven days ($F=0.7$, d.f.=4,66, $p>0.05$) or across the full 28 days ($F=2.3$, d.f.=4,66, $p>0.05$; Figure 6.2(B)).



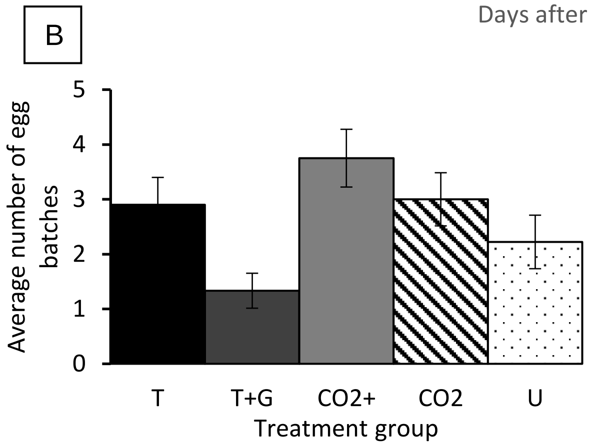


Figure 6.2. The effect of the RFID tagging process on (A) food consumption of *Deroceras reticulatum* (n=100) in four successive 7-day periods after treatment. Bars represent mean consumption (g) for each treatment group ± SE and (B) production of egg batches over the course of the 28-day experimental period following treatment by individual *Deroceras reticulatum* alive on day 28 (n=71; F=2.255, d.f.=4,66 p>0.05). Bars are mean number of batches produced ±SE. (T (Tag inserted) + CO2) Slug anaesthetised using CO2, a hole made in the body wall using the tag implanter and an RFID tag injected. (T + CO2 + G (Glue applied to insertion site)) Slug anaesthetised using CO2 and with an RFID tag injected as in treatment Tag inserted + CO2, but with the resultant hole in the body sealed with the addition of a small drop of glue (Loctite Precision Max, Loctite, USA). (CO2+) Slug anaesthetised using CO2, a hole made in the body wall using the tag implanter but without injection of an RFID tag. (CO2) Slug anaesthetised using CO2 only. (U) Untreated slug.

6.3.1.4. Locomotor behaviour

The mean distance travelled in the one-hour observation period by tagged and untagged slugs did not differ significantly in recordings made either 14, 21 or 28 days after tag insertion (F=0.3, d.f.=1, p>0.05; Figure 6.3(A)). No significant difference in the mean velocity was observed between tagged and untagged slugs in any of the experimental assessments made at 14, 21 and 28 days after tag insertion (F=0.001, d.f.=1, p>0.05; Figure 6.3(B)).

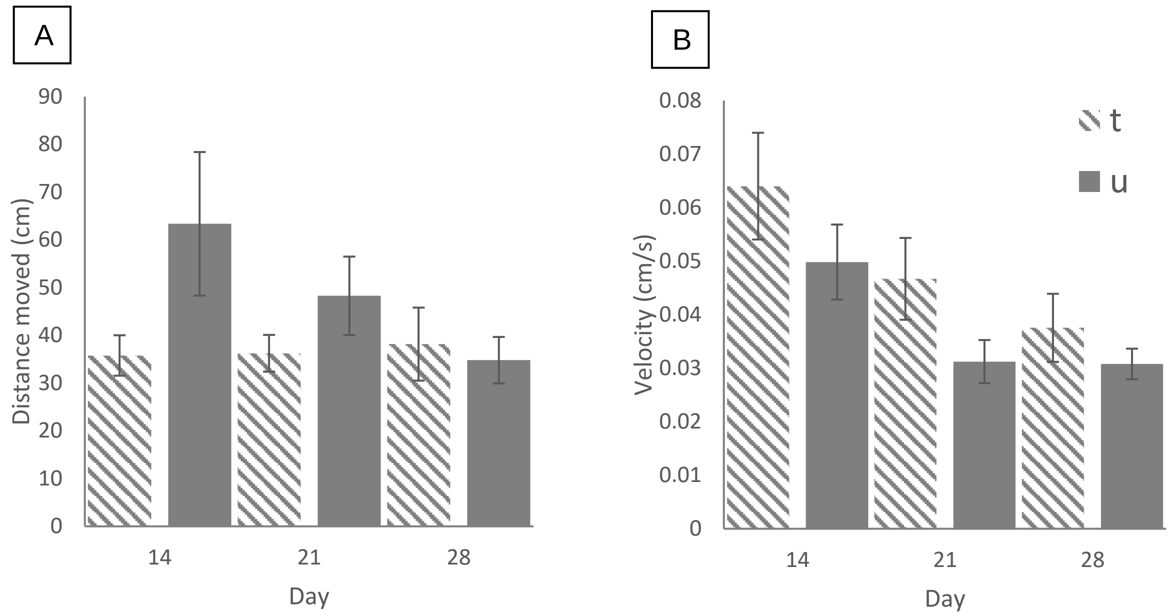


Figure 6.3. (A) Mean distance moved (cm ±SE) and (B) mean velocity (cms^{-1} ±SE) around a circular (50 cm diameter) arena by 17 tagged (t) and 17 untagged (u) slugs on day 14, 21 and 28 after tag insertion.

6.3.2. In-field tracking of slugs with implanted RFID tags

Following release into the field, slugs were readily detected when both above and below the soil surface. Tracking of slugs released in April 2017 was terminated after 38 days, whilst observations were made for 35 days following November releases.

6.3.2.1. April 2017

For the first 2 hours after release, eight of the nine slugs remained close (23.5 ±7.3 cm) to the release point (Figure 6.4(A)) and tagged slugs were observed feeding and moving over the surface. The ninth slug was not detected again after release. The first observed tagged slug feeding occurred 35 minutes after release. Two slugs were no longer visible on soil surface 1-hour post-release with all being detected within the soil horizon during assessments made at 15 hours post-release. Of the nine slugs labelled with RFID tags that were released into the field, five were regularly detected for the duration of the full five-week experimental period. The five slugs monitored throughout that period were all recorded within a short distance of the original release point (Figure 6.4(B)). The mean linear distance between detection points during the five weeks was 247 ±31.4 cm, and at the end of the experiment, the mean total displacement from the initial release point was 78.7 ±33.7 cm.

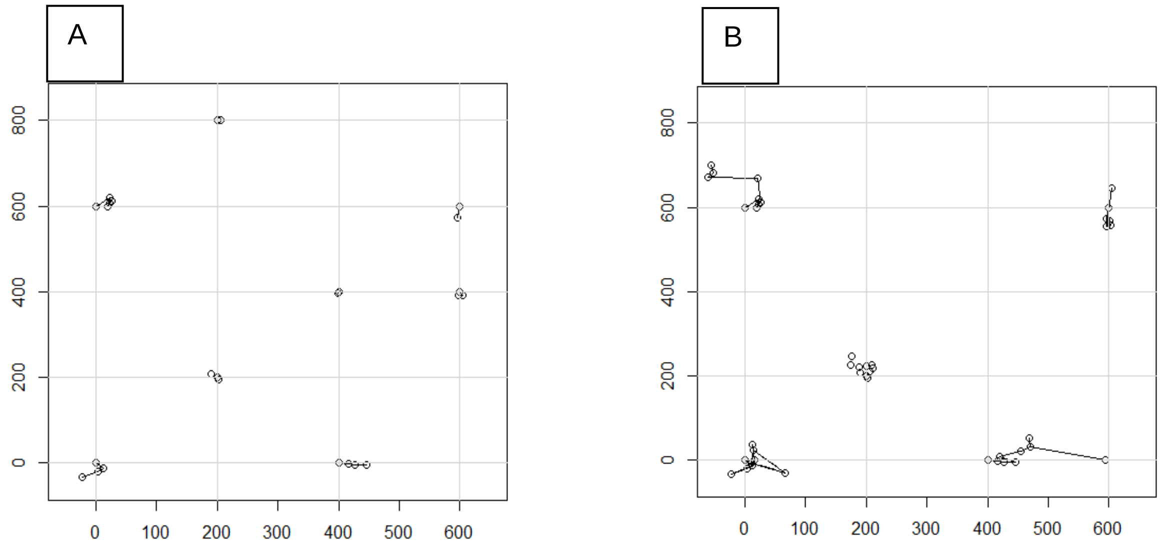


Figure 6.4. Map of *Deroceras reticulatum* movement in a field in Shropshire over (A) a two-hour period and (B) a five-week period from 5th April to 12th May 2017 using RFID technology to track and identify individuals. X and Y axis show distance (cm) from release point one (where the lines cross at 0, 0). Each circle shows a position where the slug was detected, the joining lines connect consecutive points along the slug's path but do not necessarily represent the route taken by the slug between points.

6.3.2.2. November 2017

During the three periods of intense monitoring (the night of release and following two nights) all twenty slugs remained close to their release point/first point of detection (43.3 ±10.2 cm). During the three nights of intense monitoring, slugs were observed feeding, with the first observation occurring 24, 183 and 131 minutes after sunset respectively. In total 10, 5 and 10 slugs were observed feeding on at least one occasion during the respective monitoring periods. Thereafter, of the 20 slugs released, 18 were detected regularly during the five-week experimental period (Figure 6.5). The mean linear distance between detection points during the five weeks was 514.8 ±72.0 cm, and at the end of the experiment the mean distance from the original release point was 101.9 ±24.1 cm, with the maximum distance from the original release point being 408.8 cm.

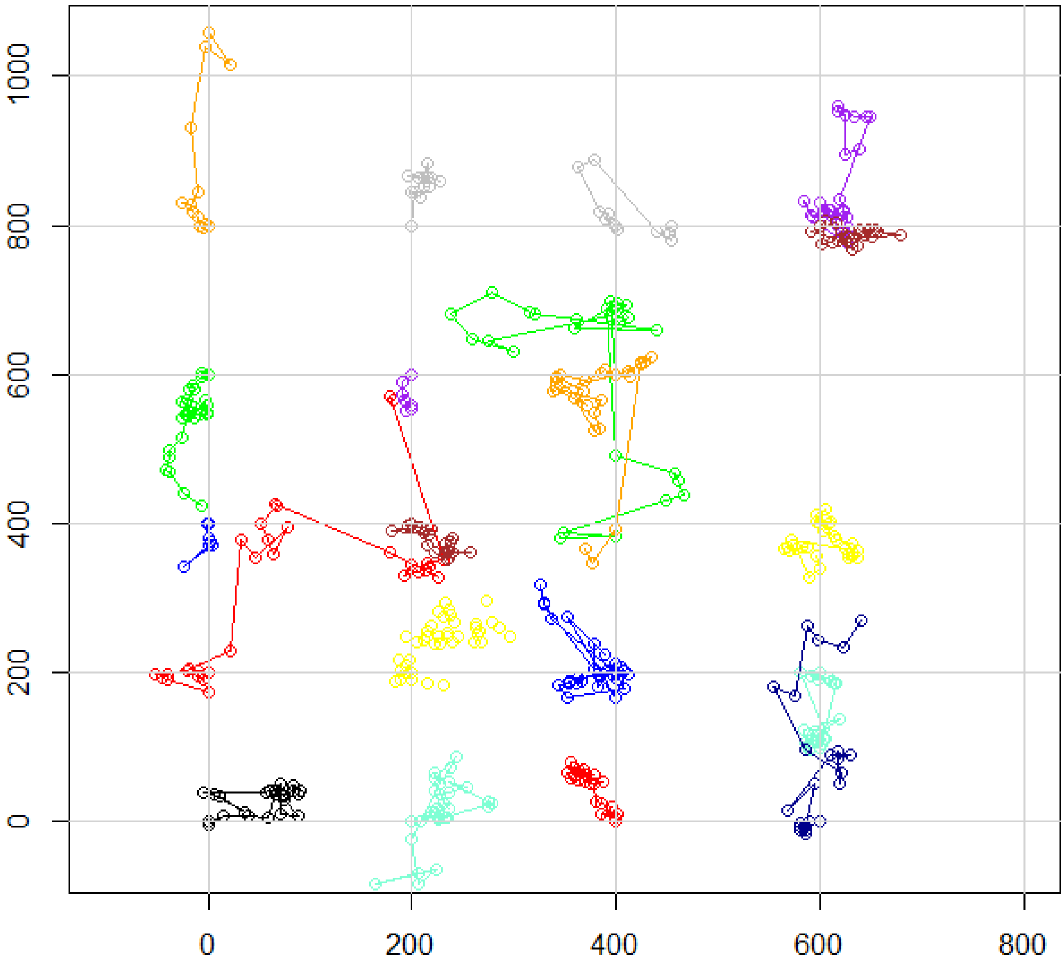


Figure 6.5. Map of *Deroceras reticulatum* movement in an 800 by 1000 cm area of a field in Shropshire over a five-week period from 15th November to 21st December 2017 using RFID technology to track and identify individuals. X and Y axis show distance (cm) from release point one (where the lines cross at 0, 0). Each circle shows a position where the slug was detected, the joining lines connect consecutive points along the slug's path but do not necessarily represent the route taken by the slug between points.

6.3.3. Effect of temperature and rainfall

6.3.3.1. April/May

Temperatures at the April/May 2017 field site (Figure 6.6(A)) were 1.2 and 2.6°C higher respectively and rainfall lower by 24.3 and 15.3 mm respectively than the 30-year average (Met Office, 2018). Within the field study period, there were 25 consecutive days with no rainfall (< 1 mm). Slug movement between daily observations showed a significant but weak correlation with temperature (Pearson's correlation; r=0.4, t=2.1, d.f.=28, $p<0.05$, R^2=0.1) but no significant correlation with rainfall (Pearson's correlation; r=-0.2, t=-1.1, d.f.=28, $p>0.05$) (Figure 6.6(A)).

6.3.3.2. November/December

The temperature (maximum and minimum within 0.6°C) during the two months of November/December (Figure 6.6(B)) was similar to the 30-year average, rainfall was lower by 9.7 mm in November and higher by 15.7 mm December (Met Office, 2018). The number of slugs active during the daily scotophase was not significantly correlated with the maximum temperature (Pearson's correlation; $r=0.4$, $t=1.9$, d.f. $=21$, $p>0.05$) but there was a significant weak correlation with daily rainfall (Pearson's correlation; $r=0.4$, $t=2.2$, d.f.$=21$, $p<0.05$). There was a period of snowfall, which remained on the ground from 8th – 15th December, coinciding with a period of low and declining slug activity (Figure 6.6(B)).

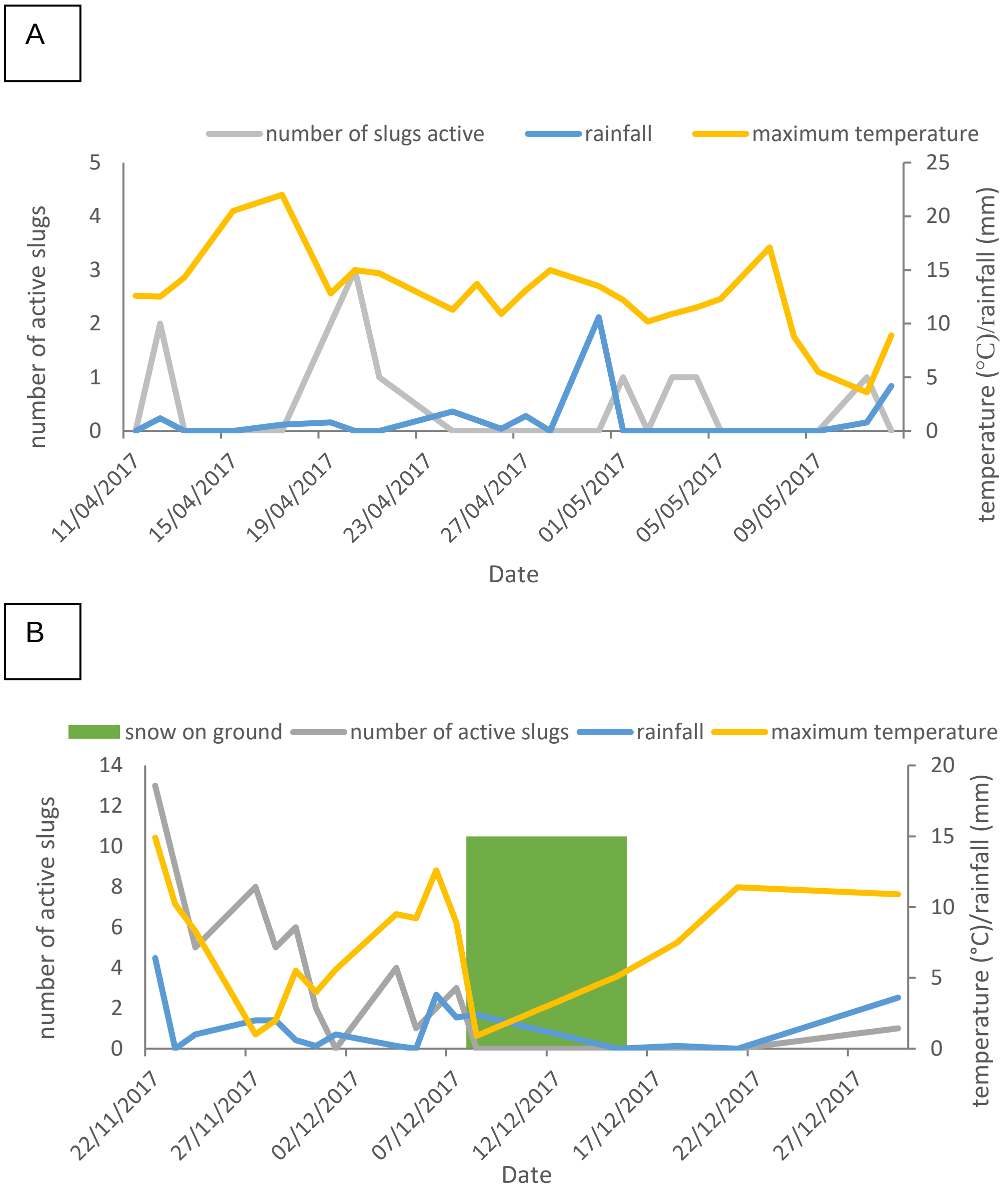


Figure 6.6. The number of active slugs overnight in relation to the maximum daily temperature (°C) and daily rainfall (mm) during (A) the five-week tracking period from 5th April 2017 and (B) the five-week tracking period from 20th November 2017.

6.4. Discussion

The reduction of pesticide usage based on application of molluscicides to areas of higher slug densities requires an understanding of the behavioural mechanisms which may lead to the formation of these patches as well as their cohesion and stability. Tracking the movement of *D. reticulatum* individuals over an extended period in the field would give an insight into these behaviours. RFID tags, unlike other techniques (Richter, 1976; Hogan and Steele, 1986; Foltan and Konvicka, 2008; Hakvoort and Schmidt, 2002) do not rely on slugs being active on the soil surface but can be used to track movement for extensive periods beneath the soil surface.

Although RFID tags have been inserted into a larger, *Arion* species (Grimm, 1996), it was previously thought that the technology could not be used in smaller slug species such as *D. reticulatum* as the relative size of RFID tags and fully grown adults would result in lethal damage being caused to the body during implanting (Foltan and Konvicka, 2008). Whilst still large in comparison to body size, advances in technology have meant smaller RFID tags are available; 8 mm tags were used in this study, compared with the 11 mm tags used by Grimm (1996). By elimination of the other components of the insertion procedure, it can be concluded that the initial effect on survival and feeding detected in the current study during the first two-week post-implantation period were due to the RFID tag itself. Slugs into which tags were implanted suffered initial increases in mortality unlike the larger *Arion* spp. tagged by Grimm (1996) and it is probable that their size made them more susceptible to damage to internal organs caused by the process, as proposed by Foltan and Konvicka (2008).

The digestive gland and crop are found on the left lateral side of a slug body (South, 1992) and damage to these organs would result in mortality occurring due to starvation over a period of days. The heart, kidney and reproductive organs are located on the right lateral side and so less are less vulnerable to damage caused by tag insertion using the procedure developed for this study; damage to these organs would be likely to lead to faster mortality than that observed in the laboratory experiments. There were no instances of immediate mortality at the time of tag insertion but increased slug mortality was recorded during a period of up to 14 days post implantation. Damage to the gustatory system may explain these observations and is supported by the finding that feeding in tagged slugs was also negatively affected. Damage to the gustatory system may limit feeding rate by reducing the capacity of the crop, leading to progressive starvation over a period of time. The ultimate recovery to normal feeding levels observed in some slugs suggests that they are able to adapt to the tag so long as the initial implantation did not cause excessive damage to internal organs or obstruct feeding to the point of starvation. No effect on egg production was identified (reflecting Grimm, 1996), supporting the proposition that organs on the right lateral side of the body are less likely to be affected by the procedure.

RFID tags were used successfully by Knop *et al.,* (2013) as a method of marking slugs in mark-recapture experiments investigating the locomotor activity of a native and invasive species. The current study extends this work, by showing that their insertion had no significant effect on slug locomotion, including the distance moved or velocity of tagged slugs. In addition, it was confirmed that the technique could be used to effectively track and record detailed behavioural characteristics pertaining to the dispersion of individual slugs in the field over a sustained period of time. The latter is a critical observation that allows the technique to be used in the field to investigate the impact of *D. reticulatum* behaviour on slug patch formation and stability. This study supports the first hypothesis "RFID tags can be implanted within the body cavity of *D. reticulatum*, without (following a defined recovery period) affecting survival, feeding, egg laying and locomotion of the individual".

Visual observations made during periods of intense monitoring in the field experiments indicated that the emergence and resumption of activity on the soil surface of naturally occurring *D. reticulatum* as dusk approached, coincided with the time of appearance of the first tagged slugs in the same area of the field, increasing confidence in the validity of the technique. Similarly, tagged slugs were found to actively feed and move over the soil surface during the night, whereas during the day they were not visible on the soil surface, consistent with published reports that slugs are more active during hours of darkness and find refuge during the day (Wareing & Bailey, 1985; South, 1992; Hommay *et al.*, 1998). The impact of both temperature and rainfall on tagged slug activity in the current study were also consistent with published findings. Mean temperatures in the April/May field experiment were close to the optimum for slug activity (movement: 17°C; feeding: 14°C; Wareing & Bailey, 1985). However, rainfall was low, which meant that soil conditions were dry throughout the experimental period, and the resultant large cracks that developed facilitated slug movement deeper into the soil than under less dry conditions (South, 1992). Significantly reduced slug activity is known to occur after extended periods of low rainfall (Choi *et al.,* 2004) and this was reflected in there being little lateral slug movement recorded on the surface, and individual slugs not being detected at every monitoring visit during the April/May period (the latter mirroring qualitative observations of naturally occurring slugs in the same field). The lack of detection, although not confirmed in this study, would suggest slugs moving vertically down the soil profile, where the temperature and moisture remains more constant, to a depth greater than the read range of the antennae. In the November/December monitoring period, the effect of rainfall was again consistent with published findings (Wareing & Bailey, 1985; Choi *et al.,* 2004), with increases in the number of active slugs coinciding with periods of rainfall. Although a period of snowfall coincided with a reduction in slug activity, some movement was still detected supporting the findings of Mellanby (1961) who found *D. reticulatum* active at temperatures as low as 0.8°C. The second hypothesis "The free movement of individual slugs in arable fields can be tracked over extended periods of time using RFID technology" was upheld by this study.

Results from both of the experimental tracking periods show that the majority (80 %) of the slugs followed had remained within a relatively short distance from their original release position at the end of the experimental periods (up to 38 days later). This suggests that longer distance dispersal of slugs within arable fields is limited, at least in established wheat crops, which may be a contributory mechanism leading to the formation of temporally and spatially stable slug patches. Formation and cohesion of the patches would be reinforced by behavioural responses that result in slugs following slime trails left by others when they are encountered (Wareing, 1986). Such mechanisms may result in the majority of slugs in winter wheat fields existing in semi-discreet groups. The low proportion of more active individuals that either rapidly dispersed from the initial release point or were otherwise found to have moved away (further than the 5 m intensive search area around the release points) from the main slug patch at the end of the observation period (approximately 20 % – 3 out of 8 slugs released in April 2017 and 2 out of 20 slugs released in November 2017), would lead to regular exchange of individuals between patches. The third hypothesis “Unless challenged by adverse conditions, individual slugs remain within a small, defined area of arable fields, with lateral restricted to less than 20 m for extended periods of time” is confirmed by this study.

Although the initial work to establish the use of RFID tags for investigating slug behaviour in the field is complete, further work is needed to develop and improve understanding of the ecological relevance of the field findings of the current study. In particular, the vertical movement through the soil profile requires further investigation in relation to weather conditions to determine the combinations of temperature and rainfall which would result in a large proportion of the population moving down the soil profile. Understanding this vertical movement would allow more efficient timings of slug control applications.

Current concerns about the impact of agricultural and horticultural practices on the natural environment have resulted in widespread recognition of the need to optimise or minimise the use of agro-chemicals in crop production (Walters and Cherrill, 2018). Whilst maintaining effective control, substantial reduction in the amount of active ingredient used to manage slug populations could be achieved by use of precision application technology to target treatments at slug patches, whilst leaving inter-patch areas untreated (Forbes *et al*., 2017). Before such an approach can be investigated, however, clear evidence that the slug patches are spatially and temporally stable is required, together with development of a commercially viable method of identifying their location and dimensions. Difficulties associated with effectively tracking *D. reticulatum* in the field have hampered investigations into slug behaviour that may affect patch formation and stability, but this study has indicated that the small spatial range of individual *D. reticulatum*, may be a major contributory factor. Research is ongoing to confirm these findings, quantify the temporal period over which patches remain cohesive, and to identify soil characteristics that define the locations in which they form.

6.5. Conclusion

In summary, RFID tagging meets the primary requirements of an effective method of tracking individual slugs, which can be identified from the unique tag numbers even when below the soil surface, and they can be tracked over periods of at least five weeks under field conditions. The data on individual slug movement collected using this technique provides evidence for a potential mechanism leading to the formation and stability of higher density slug patches in arable crops. As the pressure to reduce pesticide usage increases, improved understanding of the behaviour of *D. reticulatum* will potentially have significant economic and environmental benefits if it facilitates research into both commercially viable methods for locating slug patches in arable fields and precision application of pesticides.

Chapter 7. General discussion

7.1. Introduction

In commercial arable crops, the grey field slug (*Deroceras reticulatum*) is reported to display a discontinuous distribution whereby patches of higher numbers of slugs are distributed within areas of lower slug densities (South, 1992; Bohan *et al*., 2000a; Archard *et al*., 2004; Mueller-Warrant *et al*., 2014). In response to increasing pressure to reduce pesticide usage, this thesis investigated the potential for targeting molluscicide application to these higher density patches of *D. reticulatum*. This was achieved by determining the temporal and spatial stability of the high slug density patches, assessing the locomotory behaviour of *D. reticulatum* which underlies these discontinuous distributions observed in arable crops, and identifying some physical soil characteristics which could influence patch location. In this chapter the results and their implications are discussed in relation to commercial control of slugs, together with the limitations of the study and future work required before a commercially acceptable procedure can be developed to implement the findings.

7.1.1. Potential for patch application of molluscicides

The discontinuous distribution of slug populations in arable crops has been established in both North America and Europe, but little information is available on the temporal and spatial stability of the resultant patches of higher slug numbers. Mueller-Warrant *et al.* (2014) investigated the distribution of slugs, and reported that where numbers were highest (maximum mean number of slugs assessed using surface refuge traps of between 7.9 and 21.1 per trap) significant aggregations appeared in the same area of the field on different assessment dates, suggesting stable patches occurred. In the two field sites where the highest trap counts were below 3 slugs per trap (2.8 and 2.3), however, no stable areas of higher slug densities were detected (Mueller-Warrant *et al.,* 2014). In the current study, an analogous discontinuous distribution of slugs was observed in all fields in which the slug population was sufficiently large for statistically significant differences between high and low trap counts to be distinguished (approximate mean of 3 slugs per trap). In these fields, the patches that were defined by grid sampling were found to be spatially stable throughout the cropping season. Spatial stability is key if patch application (applying molluscicides only to areas of fields where higher slug densities occur) is to be successful, once identified growers need confidence that the area identified as having a higher density of slugs will remain in the same location throughout the susceptible crop growth stages.

Identification of stable patches was dependant on the number of slugs present suggesting that when using the trapping grid developed, a threshold for reliable patch location of approximately 3-4 slugs per trap is required to accurately locate higher density patches. In all fields with a mean trap count of 4 slugs per trap or above, spatially and temporally (across the cropping season) stable patches were identified. The difficulties associated with detecting areas of higher slug densities in the fields with low populations of surface-active slugs (in the region of 3-4 slugs per trap and below) may not indicate that in these areas they were not present, only that they could not be accurately identified with the methods of refuge trapping used in this study (Petrovskaya *et al*., 2018). In future research the patch stability detected in both the current work and that of (Mueller-Warrant *et al.,* 2014), may reduce difficulties associated with the requirement for a minimum level of slug activity on the soil surface before accurate patch location can be achieved. As patches remained in similar positions for extended periods in the crops studied, mapping may be achieved with as little as one assessment. As weather exerts a significant effect on slug behaviour (South, 1992; Choi *et al.,* 2004), days with optimum conditions could be selected for this assessment. Commercial confidence in such research may benefit from the current AHDB recommended threshold for pellet application in standing cereal crops, a mean of 4 slugs per trap from traps distributed across the field (AHDB, 2016). Future assessment of patch location in research developing patch treatment techniques would be undertaken at population levels close to those at which decisions on pellet application are made.

If patches with higher slug densities are to be used for targeted molluscicide applications, then an understanding of the biological and behavioural mechanisms underpinning their formation and coherence is essential if reliance on spatial correlation (with associated risks) is to be avoided. Investigation of slug locomotory behaviour and dispersion in the field has been hampered by their vertical distribution above and below the soil surface. Various methods have been developed for assessing slug numbers in both locations (dye marking; Hogan and Steele, 1986, surface refuge traps, soil flooding, DATs; South, 1992, UV dye; Foltan and Konvicka, 2007), but movement between these horizons has made collection of detailed data on the locomotory behaviour of individual animals over extended periods of time difficult.

The development of RFID technology offered a new method of identifying individual slugs using tags attached to the body surface or inserted into the body cavity (Grimm, 1996). The first use in the field was in a study of *Arion lusitanicus*, in which tagged slugs were released into grassland, demonstrating the potential for identifying individuals over extended periods of time (Grimm and Paill, 2001). The method was subsequently adopted for the assessment of field survival rates and locomotor activity of *Arion* spp. but in both cases as a mark-recapture technique rather than for tracking individual movement (Ryser *et al*. 2011, Knop *et al*. 2013), it was also concluded that damage caused when attaching tags to smaller species such as *D. reticulatum* may result in significant mortality or behavioural modification in survivors.

The current work extended the use of the technology by developing and testing a process by which tags could be inserted into the body cavity of *D. reticulatum* without affecting subsequent behaviour of survivors selected for use in experimental work. The method was used to investigate behavioural traits that result in the formation and coherence of slug patches in arable fields. RFID tracking in the current study yielded a higher "recapture" rate than reported by Grimm and Paill (2001) for both spring and autumn releases. This higher rate could be due to the larger read range of modern equipment; Grimm and Paill (2001) used a device with a read range up to approximately 15 cm (when there was no a barrier between tag and device) compared to a range of up to 20 cm depth of soil in this study.

Individual slugs were successfully tracked for up to 5 weeks in this study and showed that they did not return to the same refuge each night, confirming the finding of Hommay *et al.* (1998) that slugs were using the same refuge for a maximum of two consecutive nights. Despite this, the lateral dispersion of individual slugs was low. Although 20% of tracked individuals moved away and were lost after release, the mean linear displacement of the remaining 80% was only 78.7 cm (April 2017) and 101.9 cm (November 2017) from the initial release point after the 5-week period. Such a low linear displacement will result in retention of slugs in areas offering favourable soil conditions, supporting formation and cohesion of higher density patches, which in turn will be reinforced by the known slime trail following behaviour displayed by the species (Rollo and Wellington, 1981). The limited dispersion of slugs during the two tracking periods supports the work carried out looking at patch stability, providing improved understanding of a mechanism leading to areas of higher slug density remaining in the same location for extended periods of time. The low proportion of slugs that quickly moved away from the initial release point in the current field study was similar to the 27 % of slugs reported to have left the study area established in previous work (Grimm and Paill, 2001). Such dispersers may contribute to the inter-change of individuals between the discrete slug patches, and tracking these individuals, may allow conclusions to be drawn on their importance for patch cohesion and possibly the establishment of new slug patches.

7.1.2. Current limitations for patch application of molluscicides

Refuge trapping is a method of assessing surface activity of slugs rather than providing absolute population estimates (Hommay *et al*., 2003), their use for identifying the location of slug patches in a commercially viable integrated pest management system is therefore limited. Crop damage is caused by slug grazing on plants above the soil surface, and in some crops on the seeds in the soil (Glen *et al.,* 1990b; South, 1992). As slug populations are distributed between the surface and the upper horizons of the soil and the proportion in each varies with time due to the effect of a range of environmental factors, trapping methods that focus on surface activity can result in inaccurate assessments of population distribution across arable fields, particularly if reliant on a single or few assessment dates. In addition, based on the size and distribution of patches identified in the current study, models have indicated that the density of trapping points required in arable crops would preclude economic viability (Petrovskaya, 2018).

Two alternative methods for locating the areas of higher slug densities were investigated during this study. Anecdotal evidence from growers (P. Jackson, Pers. Comm.) suggest that parts of fields in which higher crop damage is more regularly observed indicate areas at generally increased risk from slug activity. Identification of such areas allows either the targeting of standard slug pellet applications to areas where they may have maximum impact, or the view is sometimes expressed by some farmers that they are best applied at a higher rate in these areas. The results from this study and the similarly weak correlation between post emergence plant density and slug numbers reported from North America (Muller-Warrant *et al*., 2014) support the conclusion that plant damage may not be a satisfactory assessment method for targeted pesticide applications. Damage from slugs before a crop emerges can prevent germination and reduce plant density (Glen *et al.,* 1993). Leaf shredding during the early growth stages in patches with high numbers of slugs can also result in lower plant densities. These reductions in plant density potentially result in greater proportional damage in such patches when compared to surrounding areas where feeding has not occurred, combined with the ability of the crop to rapidly produce new leaves when actively growing (AHDB, 2018), the value of crop damage as an indicator of slug activity is limited. Environmental factors will affect crop growth (AHDB, 2018), slug population size (Port and Port, 1986; Willis *et al.,* 2008) and different rates of feeding (Wareing and Bailey, 1986), which may also lead to variations in visible damage and weaker correlations with slug numbers.

7.1.3. Edaphic factors and areas of higher slug densities

The investigation of the possible use of soil characteristics to identify the locations in which slug patches may form was more successful and shows potential. Several soil characteristics, pH, soil moisture, bulk density (and associated porosity), soil texture and organic matter, were identified in this study as factors which could influence the distribution of slugs in arable fields and will be further investigated during a future AHDB funded project.

The importance of soil moisture for surface activity has been demonstrated by several authors (South, 1992, Shirley *et al.,* 2001; Choi *et al.,* 2004), with slugs showing a preference for damp but not waterlogged soils (Carrick, 1942; Young and Port, 1991; Glen and Symondson, 2003). Laboratory experiments in this study supported these findings, but field experiments resulted in significant differences between areas with high or low slug densities being identified at only a single site. Soil moisture levels are affected by a range of environmental conditions and are highly variable over relatively short periods of time, potentially making direct measurement inappropriate for work relating slug patch location to this factor, soil characteristics that affect water retention may be more suitable candidates for procedures identifying the location of slug patches. Factors affecting water retention include organic matter, soil texture, bulk density and infiltration.

Decomposing plant matter in the soil can affect soil properties in addition to providing a food source for slugs. Higher organic matter content can improve the water holding capacity of the soil (Franzluebbers, 2002) and improve soil structure (Boekel, 1963; Hillel, 2008) both of which are important for slugs. Slugs are unable to regulate their own body moisture and so rely on water in their environment (South, 1992), therefore, increased water retention in the soil during dry conditions would be favourable for slugs. Increased organic matter can improve soil structure, reducing bulk density of the soil and provide access to refuges within the upper soil horizons, which is important to slugs during adverse environmental conditions or for reducing the risk of predation. The results of earlier studies showing preferences for soils with higher organic matter content were not replicated in laboratory experiments in this work, however, this was possibly a result of the type of organic matter used. Significant effects demonstrated at one of the field sites and the results of other published work supports its inclusion in future work.

Soil texture (percentage of sand, silt and clay) were only assessed at a single site in this study, but a significant difference between the level of each of the three size fractions was found to occur between areas of the field with patches of higher and lower slug densities. Higher clay or silt content and lower sand content in soil will result in increased water retention making them less prone to drying (Rice, 2002; Hillel, 2008). Published evidence suggests that slugs display a preference for heavier soils with a higher clay content, partly because of the higher moisture retention characteristics (Gould, 1961; South, 1992; Ondina *et al.,* 2004; AHDB, 2016). Soil texture remains a candidate for investigation in future research.

Bulk density and associated porosity are linked to soil texture, organic matter content and compaction resulting from weather at the time of cultivation and cultivation method (Franzluebbers, 2002; Nimmo, 2004; Chaudhari *et al.,* 2013; Kalev and Toor, 2018). Soils with higher bulk densities inhibit the ability of slugs to move through the soil profile, with fewer cracks resulting in fewer available refuges and increased slug mortality (Stephenson, 1975; Kozlowski and Pallardy, 1997; Shirley *et al.*, 2001). Field experiments under the current project yielded limited evidence of significant impact of bulk density on the location of patches of higher slug density, but the factor requires further research to fully understand its role.

Infiltration rate was not found to be significantly related to slug patch location in any of the fields investigated in this study. The method used for assessment of this factor (simplified falling head method) may have contributed to this outcome due to the high variation in measurements. The alternative techniques require longer recording time, making infiltration a less suitable characteristic for determining the location of areas of higher slug densities in commercial crops.

No individual soil characteristic was strongly related to the location of slug patches in the six fields studied in this work programme. With the exception of soil pH, those factors for which a significant relationship was established with the distribution of slugs in at least one field site may affect soil moisture or the ability of slugs to move into and through the soil profile (potentially providing them a refuge from adverse environmental conditions of predators). The lack of a single factor that was related to patch location in all or most of the fields studied suggests, however, that such relationships are complex and it is likely that a combination of edaphic factors will influence the location of higher density slug patches.

7.1.4. Potential for development/uptake of targeted application for slug control

New technology for precision farming is emerging onto the market, for example, Terramap, a system that maps up to 21 different soil characteristics in arable fields has recently been released (Hutchinsons Ltd, 2016). These characteristics include several which were identified in this study as potential candidates for locating areas of higher slug densities, including pH, texture, organic matter and moisture. The system currently produces maps using 800 sampling points per hectare, a density that would create a finer grid than used in this study and so has potential for adaptation for the purpose of slug patch location. The evidence suggests that the use of precision agriculture techniques is increasing in England, a survey of over 2800 farmers in 2012 reported an increase in the use of GPS (14 to 22 %), soil mapping (14 to 20 %), variable rate applications (13 to 16 %) and yield mapping (7 to 11 %; Defra, 2013). The trend of increased uptake of precision agriculture practices continued in the 2018 survey (>2500 farmers), with 17 % of farmers having introduced new precision agriculture technologies in the previous 12 months and 7 % of farmers intending to adopt further new techniques in the following 12 months (Defra, 2019). The adoption of soil maps for the purposes of pest management has already been used for targeting nematicides for potato cyst nematode control (Perry *et al.,* 2006), PCN are a relatively immobile pest compared to many insect pests, such as aphids and beetles facilitating targeted control (Godwin and Miller, 2003). Given the adoption of precision agriculture is increasing, soil characteristics identified in this thesis are currently being mapped for other purposes (offering potential for cost sharing between different on-farm tasks) and sufficient stability of areas of higher slug densities has been confirmed, a well-researched approach for slug control may be of interest to the industry.

7.1.5 Future work

As indicated in section 7.1.3, the development of a new IPM system for slug control relies initially on an improved understanding of the interactions between the various candidate factors identified in the current study, their relationship with and impact on slug biology and behaviour, and the establishment of a combination of stable soil characteristics that strongly identifies the location of patches of higher slug density.

The work in this thesis has primarily concentrated on wheat and OSR crops, however, the combination of soil characteristics which determine the location of the higher slug densities may also apply to other crops, for example, potatoes, brassicas, lettuce, asparagus and strawberries where slug damage is also economically important (Speiser *et al.,* 2001; The Andersons Centre, 2014). Further work would be required to confirm this hypothesis. In potato crops, for example, the creation of ridges for planting alters the field environment (Stalham and Allison, 2015). Cultivation method and seed bed conditions are known to affect the number of slugs (Glen and Symondson, 2003), by creating a non-uniform surface, drier ridges with fine soil tilth and furrows with a higher moisture content. These differences would need to be investigated in potato crops in relation to slug distribution and movement to confirm whether the same method of locating patches can be used.

Between season stability was not confirmed in this study, there are several possibilities for this which need to be investigated further, such as cultivation method, effect of compaction, natural changes in the soil properties or changing distribution of natural enemies. Understanding the inter-season stability of the areas of higher slug densities remains an important area for future work.

7.1.6. Potential for pesticide reduction

Following the removal of methiocarb (HSE, 2014) from the European market and the uncertain future of metaldehyde (Appleby, 2019; Pickstone, 2019) it is increasingly important that the remaining active ingredient, ferric phosphate (Defra, 2018b) is used as sustainably as possible. Sustainable use can be promoted by optimising pesticide use through targeted application, bringing potential environmental benefits and possibly some direct savings to the grower. In addition to reducing the environmental effects of active ingredients it may also improve the cost effectiveness of alternative options such as the use of nemtatodes. Currently nematodes are not considered to be a viable option in many crops such as wheat and oilseed rape. Although unlikely to solve this issue in isolation, targeted application of the products may improve cost-benefit calculations.

The size of the slug patches detected, using a 100 m by 100 m grid, in this study varied between fields from 300 to 7000 m^2, in order to fully establish the mean size and number of these patches in arable fields, and so potential pesticide reductions, further work would need to be done.

7.2. Conclusions

Although no single individual soil factor was strongly associated with slug patch location, several candidate characteristics (organic matter, bulk density, porosity, soil texture (percentage of sand, silt and clay) and pH) varied significantly between areas of higher and lower slug densities in at least one of the fields studied. The factors identified are known to influence soil moisture through water retention, and/or the soil structure (which can provide access to refuges for slugs in the upper horizons of the soil). Future research should investigate the impact of using two or more of these functionally related factors in combination on the strength of the relationship between slug density (and slug patch location) and soil characteristics. In support of this work, research to improve our understanding of optimal ranges or critical thresholds for each characteristic should be considered. Additional research into the stability of these soil characteristics over time is also required.

References

ADAS. 2010. *A review of slug control in winter cereal and oilseed rape*. Research project final report PS2803. [ONLINE] Defra. Available at: http://sciencesearch.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=17215 [Accessed 9th June 2016]

AHDB. 2009. *Be PRECISE case studies*. [Online] AHDB. Available at: http://cereals.ahdb.org.uk/publications/2009/june/03/be-precise-case-studies.aspx [Accessed 9th October 2015]

AHDB. 2010. *Slug control*. [Online] AHDB. Available at: http://www.ahdb.org.uk/projects/slugcontrol.aspx [Accessed 9th October 2015]

AHDB. 2014. *Encyclopaedia of pests and natural enemies in field crops*. [ONLINE] AHDB. Available at: https://ahdb.org.uk/knowledge-library/encyclopaedia-of-pests-and-natural-enemies [Accessed 9th October 2015]

AHDB. 2016. *Integrated slug control*. [ONLINE] AHDB. Available at: https://cereals.ahdb.org.uk/publications/2016/april/18/integrated-slug-control.aspx [Accessed 27th July 2019]

AHDB. 2018. *Wheat growth guide*. [ONLINE] AHDB. Available at: https://cereals.ahdb.org.uk/media/185687/g66-wheat-growth-guide.pdf [Accessed 27th July 2019]

Appleby, M. 2019. *Government withdraws metaldehyde slug killer ban after "concerns raised about decision-making process"*. [ONLINE] Horticulture Week. Available at: https://www.hortweek.com/government-withdraws-metaldehyde-slug-killer-ban-concerns-raised-decision-making-process/ornamentals/article/1592601 [Accessed 24th August 2019]

Archard, G. A., Bohan, D. A., Hughes, L and Wiltshire, C. W. 2004. Spatial sampling to detect slug abundance in an arable field. *Annals of Applied Biology*, 145, pp.165-173.

Armsworth, C. G., Bohan, D. A., Powers, S. J., Glen, D. M. and Symondson, O. C. 2005. Behavioural responses by slugs to chemicals from a generalist predator. *Animal Behaviour*, 69, pp. 805-811.

Atkins, W.R.G. and Lebour, M.V. 1923. The Hydrogen ion concentration of the soil and natural water in relation to the distribution of snails. *Science Proceedings RDS*, 17, pp.234-240.

Ayre, K. 2001. Effect of predator size and temperature on the predation of *Deroceras reticulatum* (Müller) (Mollusca) by carabid beetles. *Journal of Applied Entomology*, 125, pp.389-395.

Bagarello, V., Iovino, M. and Elrick, D. 2004. A simplified falling-head technique for rapid determination of field-saturated hydraulic conductivity. *Soil Science Society of America Journal*, 68, pp.66-73.

Bagarello, V., D'Asaro, F. and Lovino, M. 2012. A field assessment of the Simplified Falling Head technique to measure the saturated soil hydraulic conductivity. *Geoderma*, 187, pp.49-58.

Ball, B.C., Campbell, D.J. and Hunter, E.A. 2000. Soil compactibility in relation to physical and organic properties at 156 sites in UK. *Soil and Tillage Research*, 57, pp.83-91.

Barker G.M. 2002. Gastropods as pests in New Zealand pastoral agriculture, with emphasis on Agriolimacidaae and Arionidae and Milacidae. In: Barker G.M. *ed Molluscs as crop pests*. Wallingford: CABI Publishing, pp. 361–423.

Batey, T. 2009. Soil compaction and soil management–a review. *Soil use and management*, 25, pp.335-345.

Bauer, A. 1974. Influence of soil organic matter on bulk density and available water capacity of soils. *Farm Research,* May/June 1974, pp.44-52.

Bellostas, N., Sørensen, A.D., Sørensen, J.C. and Sørensen, H. 2007. Genetic variation and metabolism of glucosinolates. *Advances in Botanical Research*, 45, pp.369-415.

Boekel, P. 1963. The effect of organic matter on the structure of clay soils. *Netherlands Journal of Agricultural Science*, 11, pp.250-263.

Bohan, D., Glen, D., Wiltshire, C. and Hughes, L. 2000a. Parametric intensity and spatial arrangement of the terrestrial mollusc herbivores *Deroceras reticulatum* and *Arion intermedius*. *Journal of Animal Ecology,* 69, pp.1031–1046.

Bohan, D., Bohan, A., Glen, D., Symondson, W., Wiltshire, C. and Hughes, L. 2000b. Spatial dynamics of predation by carabid beetles on slugs. *Journal of Animal Ecology,* 69, pp.367–379.

Boycott, A.E. 1934. The habitats of land Mollusca in Britain. *Journal of Ecology*, 22, pp.1-38.

Brooks, A.S., Wilcox, A., Cook, R.T., James, K.L. and Crook, M.J. 2006. The use of an alternative food source (red clover) as a means of reducing slug pest damage to winter wheat: towards field implementation. *Pest Management Science*, 62, pp.252-262.

Brouwer, C., Goffeau, A. and Heilbloem, M. 1985. *Introduction to irrigation*. Rome: Food and Agriculture Organisation of the United Nations.

Carlile, B. 2006. *Pesticide selectivity, health and the environment*. Cambridge University Press.

Carrick, R. 1942. The grey field slug *Agriolimax agrestis* L., and its environment. *Annals of Applied Biology*, 29, pp.43-55.

Castle, G.D., Mills, G.A., Gravell, A., Jones, L., Townsend, I., Cameron, D.G. and Fones, G.R. 2017. Review of the molluscicide metaldehyde in the environment. *Environmental Science: Water Research & Technology*, 3, pp.415-428.

Chase, R.W. 1981. *Potatoes*. Cooperative Extension Service, Michigan State University.

Chaudhari, P.R., Ahire, D.V., Ahire, V.D., Chkravarty, M. and Maity, S. 2013. Soil bulk density as related to soil texture, organic matter content and available total nutrients of Coimbatore soil. *International Journal of Scientific and Research Publications*, 3, pp.1-8.

Choi Y, Bohan D, Powers S, Wiltshire C, Glen D, Semenov M. 2004. Modelling *Deroceras reticulatum* (Gastropoda) population dynamics based on daily temperature and rainfall. *Agriculture, Ecosystems and Environment,* 103, pp.519–525.

Choi, Y. H., Bohan, D. A, Potting, R. P. J., Semenov, M. A. and Glen, D. M. 2006. Individual based model of slug population and spatial dynamics. *Ecological Modelling*, 190, pp.336-350.

Clark, P.J. and Evans, F.C. 1954. Distance to nearest neighbour as a measure of spatial relationships in populations. *Ecology*, 35, pp.445-453.

Clarke, A. 2014. *EU votes to ban methiocarb slug pellets*. [Online] Farmers Weekly. Available at: http://www.fwi.co.uk/arable/eu-votes-to-ban-methiocarb-slug-pellets.htm [Accessed 12 October 2015]

Clay, J. 2011. Freeze the footprint of food. *Nature*, 475, p.287.

Clements, R.O. and Murray, P. J. 1991. Comparison between defined-area slug traps and other methods of trapping slugs in cereal fields. *Crop Protection*, 10, pp.152-154.

Cohen, J.E. and Xu, M. 2015. Random sampling of skewed distributions implies Taylor's power law of fluctuation scaling. *Proceedings of the National Academy of Sciences*, 112, pp.7749-7754.

Cook, A. 1979. Homing by the slug *Limax pseudoflavus*. *Animal Behaviour,* 27, pp.545-552.

Cook, A. 1980. Field studies of homing in the pulmonate slug *Limax pseudoflavus* (Evans). *Journal of Molluscan Studies*, 46, pp.100-105.

Cook, A. 1981. A comparative study of aggregation in pulmonate slugs (genus *Limax*). *Journal of Animal Ecology*, 50, pp.703-713.

Cook, A. 2001. Behavioural ecology: on doing the right thing, in the right place at the right time. In *The Biology of Terrestrial Molluscs* (ed. G. M. Barker), pp. 447–487. CABI Publishing, Wallingford.

Cook, R., Bailey, S. and McCrohan, C. 1996. Slug preferences for winter wheat cultivars and common agricultural weeds. *Journal of Applied Ecology*, 33, pp.866-872.

Cook, R.T. 2004. The tolerance of the field slug *Deroceras reticulatum* to freezing temperatures. *Cryo Letters*, 25, pp.187-194.

Council Directive 91/414/EEC of 15 July 1991 concerning the placement of plant protection products on the market. [Online] *Official Journal of the European Union* L 230/1. Available at: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31991L0414&from=en [Accessed 9th June 2016]

Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. [Online]. *Official Journal of the European Union* L 330/32. Available at: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31998L0083&from=EN [Accessed 27th August 2016]

Council Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. [Online] *Official Journal of the European Union* L 327/1. Available at: http://eur-lex.europa.eu/resource.html?uri=cellar:5c835afb-2ec6-4577-bdf8-756d3d694eeb.0004.02/DOC_1&format=PDF [Accessed 9th June 2016]

Council Directive 2006/118/EC of the of the European Parliament and of the Council of 12 December 2006 on the protection of groundwater against pollution and deterioration. [ONLINE] *Official Journal of the European Union* L 372/19. Available at: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:372:0019:0031:EN:PDF [Accessed 3rd September 2019]

Council Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for community action to achieve the sustainable use of pesticides. [Online] *Official Journal of the European Union* L 309/71. Available at: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02009L0128-20091125&from=EN [Accessed 27th August 2016]

Cranfield University 2019. *The Soils Guide.* [ONLINE] Cranfield University, UK. Available at: www.landis.org.uk. [Accessed 16th May 2019]

Darrouzet-Nardi, A. 2018. *Package 'hotspots'*. [Online] The comprehensive R archive network. Available at: https://cran.r-project.org/web/packages/hotspots/hotspots.pdf [Accessed 16th June 2019]

Davies, M. and Blackwell, J. 2007. Energy saving through trail following in a marine snail. *Proceedings of the Royal Society B: Biological Sciences,* 274, pp.1233-1236.

Defra. 2006. *Code of practice for using plant protection products.* [ONLINE] UK Government. Available at: http://www.hse.gov.uk/pesticides/resources/C/Code_of_Practice_for_using_Plant_Protection_Products_-_Complete20Code.pdf [Accessed 2nd September 2019]

Defra. 2013. *Farm Practices Survey Autumn 2012 – England.* [ONLINE] National Statistics Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/181719/defra-stats-foodfarm-environ-fps-statsrelease-autumn2012edition-130328.pdf [Accessed 9th August 2019]

Defra. 2018a. *The guide to cross compliance in England 2019*. [ONLINE] UK Government. Available at: https://www.gov.uk/guidance/cross-compliance-2019 [Accessed 2nd September 2019]

Defra. 2018b. *Restrictions on the use of metaldehyde to protect wildlife*. [Online] UK Government. Available at: https://www.gov.uk/government/news/restrictions-on-the-use-of-metaldehyde-to-protect-wildlife [Accessed 2nd May 2019]

Defra. 2019. *Farm Practices Survey 2018 – England.* [ONLINE] National Statistics. Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/792165/fps-general-statsnotice-4apr19.pdf [Accessed 9th August 2019]

Deleon, L., Brewer, M.J., Esquivel, I.L. and Halcomb, J. 2017. Use of a geographic information system to produce pest monitoring maps for south Texas cotton and sorghum land managers. *Crop Protection*, 101, pp.50-57.

Denny, M.W. 1981. A quantitative model for the adhesive locomotion of the terrestrial slug, *Ariolimax columbianus*. *Journal of Experimental Biology*, 91, pp.195-217.

Dexter, A.R. 2004. Soil physical quality: Part I. Theory, effects of soil texture, density, and organic matter, and effects on root growth. *Geoderma*, 120, pp.201-214.

Dörler, D., Scheucher, A. and Zaller, J.G. 2019. Efficacy of chemical and biological slug control measures in response to watering and earthworms. *Scientific Reports*, 9, pp.2954.

Eaton, R.J., Barbercheck, M., Buford, M. and Smith, W. 2004. Effects of organic matter removal, soil compaction, and vegetation control on Collembolan populations. *Pedobiologia*, 48, pp.121-128.

Environment Agency. 2012. *Freshwater Eutrophication* [ONLINE] Environment Agency. Available at: https://www.rivermease.co.uk/wp-content/uploads/2015/07/Freshwater_Eutrophication_Briefing_Note_December_20121.pdf [Accessed 9th August 2019]

Evans, A and Barker, A. 2017. *Final Report Evaluation of the efficacy and timing of molluscicide treatments in potato crops 2015 & 2016. Ref: 11120025.* [ONLINE] AHDB Potatoes. Available at: https://potatoes.ahdb.org.uk/publications/11120025-evaluation-efficacy-and-timing-molluscicide-treatments [Accessed 20th April 2019]

Ferguson, A.W., Klukowski, Z., Walczak, B., Clark, S.J., Mugglestone, M.A., Perry, J.N. and Williams, I.H. 2003. Spatial distribution of pest insects in oilseed rape: implications for integrated pest management. *Agriculture, Ecosystems & Environment*, 95, pp.509-521.

Ferguson, C. M. and Hanks, C. B. 1990. Evaluation of defined-area trapping for estimating the density of the field slug, *Deroceras reticulatum* (Müller). *Annals of Applied Biology,* 117, pp.451-454.

Foltan, P. and Konvicka, M. 2008. A new method for marking slugs by ultraviolet-fluorescent dye. *Journal of Molluscan Studies*, 74, pp.293–297.

Forbes, E., Back, M., Brooks, A., Petrovskaya, N., Petrovskii, S., Pope, T. and Walters, K. F.A. 2017. Sustainable management of slugs in commercial fields: assessing the potential for targeting control measures. *Aspects of Applied Biology*, 134, pp.89-96.

Franzluebbers, A. 2002. Water infiltration and soil structure related to organic matter and its stratification with depth. *Soil and Tillage Research*, 66, pp.197–205.

Fullen, M.A. 1985. Compaction, hydrological processes and soil erosion on loamy sands in east Shropshire, England. *Soil and Tillage Research*, 6, pp.17-29.

Garthwaite, D., Barker, L., Laybourn, R., Huntly, A., Parrish, G. P., Hudson, S. and Thygesen, H. 2015. *Pesticide usage survey report 263; Arable crops in the UK 2014.* [ONLINE] Fera. Available at: https://secure.fera.defra.gov.uk/pusstats/surveys/documents/arable2014v2.pdf [Accessed 20th April 2019]

Garthwaite, D., Barker, I., Ridley, L., Mace, A., Parrish, G., MacArthur, R. and Lu, Y. 2018. *Pesticide Usage Survey Report 271; Arable crops in the UK 2016*. [ONLINE] Fera. Available at: https://secure.fera.defra.gov.uk/pusstats/surveys/documents/arable2016-v9.pdf [Accessed 20th April 2019]

Gelperin, A. 1974. Olfactory basis of homing behaviour in the giant garden slug, *Limax maximus. Proceedings of the National Academy of Sciences,* 71, pp. 966-970.

Getz, L. L. 1959. Notes on the Ecology of Slugs: *Arion circumscriptus*, *Deroceras reticulatum*, and *D. laeve*. *American Midland Naturalist*, 61, p.485.

Glass, C. R., Boatman, N. D., Brown, C. B., Garthwaite, D. and Thomas, M. 2006. *Evaluation of the performance of the Voluntary Initiative for pesticides in the UK. Report number P3OG1001*. [ONLINE] HSE. Available at: http://www.hse.gov.uk/pesticides/resources/P/performance_of_the_vi_for_pesticides.pdf [Accessed 12th May 2019]

Glen, D.M., Milsom, N.F. and Wiltshire, C.W., 1989. Effects of seed-bed conditions on slug numbers and damage to winter wheat in a clay soil. *Annals of Applied Biology*, 115, pp.177-190.

Glen, D.M., Jones, H. and Fieldsend, J.K. 1990a. Damage to oilseed rape seedlings by the field slug *Deroceras reticulatum* in relation to glucosinolate concentration. *Annals of Applied Biology*, 117, pp.197-207.

Glen, D.M., Milsom, N.F. and Wiltshire, C.W. 1990b. Effect of seed depth on slug damage to winter wheat. *Annals of Applied Biology*, 117, pp.693-701.

Glen, D.M., Spaull, A., Mowat, D., Green, D. and Jackson, A. 1993. Crop monitoring to assess the risk of slug damage to winter wheat in the United Kingdom. *Annals of Applied Biology*, 122, pp. 161-172.

Glen, D.M., El Titi, A., Katzur, K., Buchs, W., Kreye, H., Ulber, B., Worz, M. 2003. *Further development of farmer-friendly methods for estimating slug infestation incidence in soil and damage risk to oilseed rape. Final Project Report*. [Online] UFOP. Available at: https://www.ufop.de/files/9013/3922/7287/FurthrFarmerFriendly.pdf [Accessed 12th May 2019]

Glen, D.M. and Symondson, W.O. 2003. Influence of soil tillage on slugs and their natural enemies. In: El Titi, *ed.* A *Soil tillage in agroecosystems*. Florida: CRC Press. Pp.207-227.

Glen, D.M., Wiltshire, C.W. and Bohan, D.A. 2006a. *Paper 7: Abundance and vertical distribution of slugs in soil following cultivation in Integrated slug control in arable crops: Risk assessment, trapping, agronomy and chemical control. Project report no. 393*. [ONLINE] HGCA. Available at: https://pdfs.semanticscholar.org/1711/ee55441aa4782c85118661e654a674166d5a.pdf [Accessed 16th July 2019]

Glen, D.M., Wiltshire, C.W. and Bohan, D.A. 2006b. *Paper 6: Slug damage to winter wheat seeds in relation to slug body weight in Integrated slug control in arable crops: Risk assessment, trapping, agronomy and chemical control. Project report no. 393.* [ONLINE] HGCA. Available at: https://pdfs.semanticscholar.org/1711/ee55441aa4782c85118661e654a674166d5a.pdf [Accessed 5th August 2019]

Godwin, R.J. and Miller, P.C.H. 2003. A review of the technologies for mapping within-field variability. *Biosystems Engineering*, 84, pp.393-407.

Gould, H. J. 1961. Observations on slug damage to winter wheat in East Anglia, 1957-1959. *Plant Pathology*, 10, pp.142–146.

Grimm, B. 1996. A new method for individually marking slugs (*Arion lusitanicus* (Mabille)) by magnetic transponders. *Journal of Molluscan Studies*, 62, pp.477-482.

Grimm, B. and Paill, W. 2001. Spatial distribution and home-range of the pest slug *Arion lusitanicus* (Mollusca: Pulmonata). *Acta Oecologica*, 22, pp.219-227.

Gupta, S. and Larson, W.E. 1979. Estimating soil water retention characteristics from particle size distribution, organic matter percent, and bulk density. *Water resources research*, 15, pp.1633-1635.

Gupta, S.C., Sharma, P.P. and DeFranchi, S.A. 1989. Compaction effects on soil structure. *Advances in Agronomy*, 42, pp.311-338

Haghverdi, A., Leib, B.G., Washington-Allen, R.A., Ayers, P.D. and Buschermohle, M.J., 2015. Perspectives on delineating management zones for variable rate irrigation. *Computers and Electronics in Agriculture*, 117, pp.154-167

Hakvoort, S. and Schmidt, O. 2002. ^{15}N stable isotope labelling of slugs (Gastropoda: Pulmonata). *Annals of Applied Biology*, 141, pp.275-281.

Hamilton, P.A. and Wellington, W.G. 1981. The effects of food and density of the movement of *Arion ater* and *Ariolimax columbianus* (Pulmonata: Stylommatophora) between habitats. *Researches on Population Ecology*, 23, pp.299-308.

Health and Safety Executive. 2014. *Withdrawal of Plant protection Products in the United Kingdom; Plant Protection Products Regulation (EC) no 1107/2009; Step 1 withdrawal notice – methiocarb.* [Online] UK Government. Available at: https://secure.pesticides.gov.uk/pestreg/getfile.asp?documentid=23563 [Accessed 20th September 2017]

Henry, M., Beguin, M., Requier, F., Rollin, O., Odoux, J., Aupinel, P., Aptel, J., Tchamitchian, S. and Decourtye, A. 2012. A common pesticide decreases foraging success and survival in honey bees. *Science*, 336, pp.348-350.

Hillel, D. 2008. *Soil in the Environment.* London: Elsevier.

Hillocks, R. 2012. Farming with fewer pesticides: EU pesticide review and resulting challenges for UK agriculture. *Crop Protection*, 31, pp.85-93.

Hogan, J. and Steele, G. 1986. Dye-marking slugs. *Journal of Molluscan Studies*, 52, pp.138-143.

Hommay, G., Lorvelec, O. and Jacky, F. 1998. Daily activity rhythm and use of shelter in the slugs *Deroceras reticulatum* and *Arion distinctus* under laboratory conditions. *Annals of Applied Biology,* 132, pp.167-185.

Hommay, G., Kienlen, J.C., Gertz, C. and Hill, A. 2001. Growth and reproduction of the slug Limax valentianus Firussac in experimental conditions. *Journal of Molluscan Studies*, 67, pp.191-207.

Hommay, G., Kienlen, J.C., Jacky, F. and Gertz, C. 2003. Daily variation in the number of slugs under refuge traps. *Annals of Applied Biology*, 142, pp.333-339.

Howlett, S., Port, G., Craig, A. and Bohan, D. A. 2005. Estimation of surface active slug populations using refuge traps. *Insect Pathogens and Entomoparasitic Nematodes,* 28, pp.53-109.

Hudson, B.D. 1994. Soil organic matter and available water capacity. *Journal of Soil and Water Conservation*, 49, pp.189-194.

Hutchinsons Ltd. 2016. *TerraMap*. [ONLINE] Hutchinsons Ltd. Available at: https://www.omniaprecision.co.uk/terramap/ [Accessed 8th August 2019]

Iglesias, J., Castillejo, J. and Castro, R. 2003. The effects of repeated applications of the molluscicide metaldehyde and the biocontrol nematode *Phasmarhabditis hermaphrodita* on molluscs, earthworms, nematodes, acarids and collembolans: a two-year study in north-west Spain. *Pest Management Science*, 59, pp.1217-1224.

Jessop, H. 1969. The effects of simulated slug damage on the yield of winter wheat. *Plant Pathology*, 18, pp.172-175.

John Innes Centre. 2019. *Grey field slug.* [ONLINE] John Innes Centre. Available at: https://www.jic.ac.uk/research-impact/scientific-facilities/entomology-and-insectary/slugwatch/slug-identification-guide/grey-field-slug-2/ Accessed 4th November 2019]

Kalev, S and Toor, G. 2018. The Composition of Soils and Sediments. In: Torok, B and Dransfield, T. *ed. Green Chemistry: an inclusive approach.* Massachusetts: Elsevier. Pp.339-357.

Keller, T. and Håkansson, I. 2010. Estimation of reference bulk density from soil particle size distribution and soil organic matter content. *Geoderma*, 154, pp.398-406.

Kemp, N.J. and Newell, P.F. 1987. Slug damage to the flag leaves of winter wheat. *Journal of Molluscan Studies*, 53, pp.109-111.

Kennedy, T.F., Connery, J., Fortune, T., Forristal, D. and Grant, J. 2013. A comparison of the effects of minimum-till and conventional-till methods, with and without straw incorporation, on slugs, slug damage, earthworms and carabid beetles in autumn-sown cereals. *Journal of Agricultural Science*, 151, pp.605-629.

Kettler, T.A., Doran, J.W. and Gilbert, T.L., 2001. Simplified method for soil particle-size determination to accompany soil-quality analyses. *Soil Science Society of America Journal*, 65, pp.849-852.

Khanal, S., Fulton, J. and Shearer, S., 2017. An overview of current and potential applications of thermal remote sensing in precision agriculture. *Computers and Electronics in Agriculture*, 139, pp.22-32.

Knop, E., Rindlisbacher, N., Ryser, S. and Gruebler, M. 2013. Locomotor activity of two sympatric slugs: implications for the invasion success of terrestrial invertebrates, *Ecosphere*. 4: article 92.

Kozlowski, J. and Sionek, R. 2000. The rate of egg laying and hatching of the slug *Arion lusitanicus* Mabille, a pest of arable crops. *Journal of Plant Protection Research*, 40, pp.162-167.

Kozlowski, J. and Jaskulska, M. 2014. The effect of grazing by the slug *Arion vulgaris*, *Arion rufus* and *Deroceras reticulatum* (Gastropoda: Pulmonata: Stylommatophora) on leguminous plants and other small-area crops. *Journal of Plant Protection Research.* 54, pp.258-266.

Kozlowski, T.T. and Pallardy, S.G. 1997. *Growth control in woody plants*. Elsevier. Pp.277-279.

Kromp, B. 1999. Carabid beetles in sustainable agriculture: a review on pest control efficacy, cultivation impacts and enhancement. In *Invertebrate biodiversity as bioindicators of sustainable landscapes*. Elsevier. Pp.187-228.

Langan, A.M. and Shaw, E.M. 2006. Responses of the earthworm *Lumbricus terrestris* (L.) to iron phosphate and metaldehyde slug pellet formulations. *Applied Soil Ecology*, 34, pp.184-189.

Lewis, D.B. and Magnuson, J.J. 2000. Landscape spatial patterns in freshwater snail assemblages across Northern Highland catchments. *Freshwater Biology*, 43, pp.409-420.

Liaghat, S. and Balasundram, S.K., 2010. A review: The role of remote sensing in precision agriculture. *American Journal of Agricultural and Biological Sciences*, 5, pp.50-55.

Lindblom, J., Lundström, C., Ljung, M. and Jonsson, A. 2017. Promoting sustainable intensification in precision agriculture: review of decision support systems development and strategies. *Precision Agriculture*, 18, pp.309-331.

Mahlein, A.K., Oerke, E.C., Steiner, U. and Dehne, H.W. 2012. Recent advances in sensing plant diseases for precision crop protection. *European Journal of Plant Pathology*, 133, pp.197-209.

Mahlein, A.K., 2016. Plant disease detection by imaging sensors–parallels and specific demands for precision agriculture and plant phenotyping. *Plant Disease*, 100, pp.241-251.

Mair, J. and Port, G.R. 2001. Predation by the carabid beetles *Pterostichus madidus* and *Nebria brevicollis* is affected by size and condition of the prey slug *Deroceras reticulatum. Agricultural and Forest Entomology*, 3, pp.99-106.

Mark, O. 2014. *Eight of the best slug pelleters on test*. [ONLINE] Farmers Weekly. Available at: https://www.fwi.co.uk/machinery/eight-of-the-best-slug-pelleters-on-test#tabs-8 [Accessed 3rd September 2019]

McLaughlin, J.R. and Syers, J.K. 1978. Stability of ferric phosphates. *Journal of Soil Science*, 29, pp.499-504.

Mellanby, K. 1961. Slugs at low temperatures. *Nature,* 189, pp.944.

Met Office. 2018. *Newport (Salop) Climate*. [Online] MetOffice. Available at: https://www.metoffice.gov.uk/public/weather/climate/gcqk28u76 [Accessed 6th November 2018]

Metaldehyde Stewardship Group. 2019. *MSG Stewardship Guidelines*. [ONLINE] MSG. Available at: https://www.getpelletwise.co.uk/home/msg-guidelines/ [Accessed 16th July 2019]

Meir, E. and Fagan, W.F. 2000. Will observation error and biases ruin the use of simple extinction models? *Conservation Biology*, 14, pp.148-154.

Ministry of Agriculture. 1986. *The Analysis of Agricultural Materials.* Third edition. London: Her Majesty's Stationary Office.

Mueller-Warrant, G., Anderson, N., Sullivan, C., Whittaker, G. and Trippe, K. 2014. Can knowledge of spatial variability in slug populations help improve stand establishment? *Seed Production Research, Oregon State University* 151, pp.4–13

Nash, M. A., Thomson, L. J., Horne, P. A. and Hoffmann, A. A. 2008. *Notonomus gravis* (Chaudoir) (Coleoptera: Carabidae) predation of *Deroceras reticulatum* (Müller) (Gastropoda: Agriolimacidae), an example of fortuitous biological control. *Biological Control*, 47, pp. 328-334.

Ng, T., Saltin, S., Davies, M., Johannessen, K., Stafford, R. and Williams, G. 2013. Snails and their trails: the multiple functions of trail-following in gastropods. *Biological Reviews*, 88, pp.683-700.

Nicholls, C. J. 2014. *Implications of not controlling slugs in oilseed rape and wheat in the UK. Research review: No. 79.* [ONLINE] AHDB. Available at: https://cereals.ahdb.org.uk/publications/2014/march/10/implications-of-not-controlling-slugs-in-oilseed-rape-and-wheat-in-the-uk.aspx [Accessed 7th February 2016]

Nimmo, J.R. 2004. *Porosity and pore size distribution.* In: Hillel, D. *ed*., Encyclopedia of Soils in the Environment, vol. 3. London: Elsevier. Pp.295-303.

Oberholzer, F. and Frank, T. 2003. Predation by the carabid beetles *Pterostichus melanarius* and *Poecilus cupreus* on slugs and slug eggs. *Biocontrol Science and Technology*, 13, pp.99-110.

Ondina, P., Hermida, J., Outeiro, A. and Mato, S. 2004. Relationships between terrestrial gastropod distribution and soil properties in Galicia (NW Spain). *Applied Soil Ecology*, 26, pp.1-9.

Papadopoulos, N.T., Katsoyannos, B.I. and Nestle, D. 2003. Spatial autocorrelation analysis of a *Ceratitis capitata* (Diptera: Tephritidae) adult population in a mixed deciduous fruit orchard in northern Greece. *Environmental Entomology*, 32, pp.319-326.

Patel, S. 2018. *Slug behavioural responses to different organic matter substrates*. CREST project report, FR-865789081. [Unpublished]

Perry, C., Vellidis, G., Sullivan, D.G., Rucker, K. and Kemerait, R. 2006, January. Predicting nematode hotspots using soil EC data. In *Proc. 2006 Beltwide Cotton Conference*. National Cotton Council San Antonio, TX.

Petrovskaya, N., Petrovskii, S. and Murchie, A.K. 2012. Challenges of ecological monitoring: estimating population abundance from sparse trap counts. *Journal of The Royal Society Interface*, 9, pp.420-435.

Petrovskaya, N.B., Forbes, E., Petrovskii, S.V., Walters, K.F.A. 2018. Towards the development of a more accurate monitoring procedure for invertebrate populations, in the presence of an unknown spatial pattern of population distribution in the field. *Insects*, 9, p.29

Pickstone, S. 2018. *DEFRA to reconsider future use of metaldehyde after legal challenge.* [ONLINE] Haymarket Media Group Ltd. Available at: https://www.endsreport.com/article/1592611/defra-reconsider-future-use-metaldehyde-legal-challenge [Accessed 24th August 2019]

Pope, T., Gundalai, E., Elliott, L., Blackshaw, R., Hough G., Wood, A., Bennison, J., Prince, G. and Chandler, D. 2015. Recording the movement of adult vine weevil within a strawberry crop using radio frequency identification tags. *Journal of Berry Research*, 5, pp.197-206.

Port, C.M., and Port, G. R. 1986. The biology and behaviour of slugs in relation to crop damage and control. *Agricultural Zoology Reviews,* 1, pp.255-299.

Purvis, G. and Bannon, J.W. 1992. Non-target effects of repeated methiocarb slug pellet application on carabid beetle (Coleoptera: Carabidae) activity in winter-sown cereals. *Annals of Applied Biology*, 121, pp.401-422.

R Core Team. 2013. *R: A language and environment for statistical computing. R Foundation for statistical computing, Vienna, Austria.* [ONLINE] The R Foundation. Available at: http://www.R-project.org/ [Accessed 23rd August 2019]

Rae, R., Robertson, J. and Wilson, M. 2005. Modern developments in slug control. *Outlooks on Pest Management*, 16, p.27.

Rae, R.G., Verdun, C., Grewal, P.S., Robertson, J.F., Wilson, M.J. 2007. Biological control of terrestrial molluscs using *Phasmarhabditis hermaphrodita* – progress and prospects. Pest Management Science, 63, pp.1153-1164.

Ramsden, M., Kendall, S.L., Ellis, S.A. and Berry, P.M. 2017. A review of economic thresholds for invertebrate pests in UK arable crops. *Crop Protection.* 96, pp.30-43.

Regulation (EC) No. 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing council directives 79/117/EEC and 91/414/EEC. [ONLINE] *Official Journal of the European Union* L 309/1. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32009R1107 [Accessed 3rd September 2019]

Regulation (EC) No.1185/2009 of the European Parliament and of the Council of 25 November 2009 concerning statistics on pesticides. [ONLINE] *Official Journal of the European Union* L 324/1. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32009R1185 [Accessed 3rd September 2019]

Regulation (EC) No. 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. [ONLINE] *Official Journal of the European Union* L 353/1. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32008R1272 [Accessed 3rd September 2019]

Regulation (EC) No. 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. [ONLINE] *Official Journal of the European Union* L 70/1. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32005R0396&from=EN [Accessed 3rd September 2019]

Reise, H. 2007. A review of mating behaviour in slugs of the genus *Deroceras* (Pulmonata: Agriolimacidae). *American Malacological Bulletin* 23, pp.137–156.

Rhodes, E.M., Liburd, O.E. and Grunwald, S. 2011. Examining the spatial distribution of flower thrips in southern highbush blueberries by utilizing geostatistical methods. *Environmental Entomology*, 40, pp.893-903.

Rice, T. 2002. Importance of soil texture to vineyard management. *Practical Winery and Vineyard Journal,* March/April 2002, [ONLINE] Practical Winery. Available at: https://digitalcommons.calpoly.edu/cgi/viewcontent.cgi?referer=https://scholar.google.co.uk/&httpsredir=1&article=1031&context=ersc_fac [Accessed 12th April 2019]

Richter, K. 1976. A method for individually marking slugs. *Journal of Molluscan Studies,* 42, pp.146-151.

Rollo, D. and Wellington, W. 1981. Environmental orientation by terrestrial mollusca with particular reference to homing behaviour. *Canadian Journal of Zoology,* 59, pp.225-2339.

Rollo, C. D. 1983. Consequences of competition on the reproduction and mortality of three species of terrestrial slugs. *Researches on Population Ecology*, 25, pp.20–43.

Roussel, J., Haro, A. and Cunjak, R.A. 2000. Field test of a new method for tracking small fishes in shallow rivers using passive integrated transponder (PIT) technology. *Canadian Journal of Fisheries and Aquatic Sciences*, 57, pp.1326-1329.

Rowson, B., Turner, J., Anderson, R and Symondson, B. 2014. *Slugs of Britain and Ireland; Identification, understanding and control.* Telford: FSC Publications.

Ryser, S., Rindlisbacher, N., Gruebler, M. and Knop, E. 2011. Differential survival rates in a declining and invasive farmland gastropod species. *Agriculture, Ecosystems and Environment*, 144, pp.302-307.

Schley, D. and Bees, M.A. 2002. A discrete slug population model determined by egg production. *Journal of Biological Systems*, 10, pp.243-264.

Schley, D. and Bees, M. A. 2003. Delay dynamics of the slug *Deroceras reticulatum*, an agricultural pest. *Ecological Modelling*, 162, pp.177-198.

Schmitz, O.J. and Barton, B.T. 2014. Climate change effects on behavioural and physiological ecology of predator–prey interactions: implications for conservation biological control. *Biological Control*, *75*, pp.87-96.

Sciarretta, A. and Trematerra, P. 2014. Geostatistical tools for the study of insect spatial distribution: practical implications in the integrated management of orchard and vineyard pests. *Plant Protection Science*, 50, pp.97-110.

Sekulic, G. and Rempel, C. 2016. Evaluating the role of seed treatments in canola/oilseed rape production: Integrated pest management, pollinator health, and biodiversity. *Plants,* 5, pp. 32.

Shirley, M.D., Rushton, S.P., Young, A.G. and Port, G.R. 2001. Simulating the long-term dynamics of slug populations: a process-based modelling approach for pest control. *Journal of Applied Ecology*, 38, pp.401-411.

Skinner, R.J. and Todd, A.D. 1998. Twenty-five years of monitoring pH and nutrient status of soils in England and Wales. *Soil Use and Management*, 14, pp.162-169.

Speiser, B. 1999. Molluscicidal and slug-repellent properties of anaerobically digested organic matter. *Annals of Applied Biology*, 135, pp.449-455.

Speiser, B., Glen, D., Piggott, S., Ester, A., Davies, K., Castillejo, J. and Coupland, J. 2001. *Slug damage and control of slugs in horticultural crops*. [ONLINE] CORE. Available at: https://core.ac.uk/download/pdf/10919097.pdf [Accessed 6th August 2019]

Speiser, B. and Kistler, C. 2002. Field tests with a molluscicide containing iron phosphate. *Crop Protection*, 21, pp.389-394.

South, A. 1965. Biology and ecology of *Agriolimax reticulatus* (Müller) and other slugs: spatial distribution. *Journal of Animal Ecology.* 34, pp.403-417.

South, A. 1982. A comparison of the life cycles of *Deroceras reticulatum* (Müller) and *Arion intermedius* normand (pulmonata: stylommatophora) at different temperatures under laboratory conditions. *Journal of Molluscan Studies*, 48, pp.233–244.

South, A. 1989. A comparison of the life cycles of the slugs *Deroceras reticulatum* (Müller) and *Arion intermedius* normand on permanent pasture. *Journal of Molluscan Studies*, 55, pp.9–22.

South, A. 1992. *Terrestrial slugs: Biology, Ecology and Control.* London: Chapman & Hall.

Stalham, M and Allison, M. 2015. *Improving cultivation practices in potatoes to increase window of workability and soil structural stability. Report no. 2015/6*. [ONLINE] AHDB. Available at: https://potatoes.ahdb.org.uk/sites/default/files/publication_upload/201211%20Soil%20Workability%20R452_0.pdf [Accessed 6th August 2019]

Stephenson, J.W. 1967. The distribution of slugs in a potato crop. *Journal of Applied Ecology*, 4, pp.129-135.

Stephenson, J.W. 1975. Laboratory observations on the effect of soil compaction on slug damage to winter wheat. *Plant Pathology*, 24, pp.9-11.

Symondson, W.O.C., Glen, D.M., Wiltshire, C.W., Langdon, C.J. and Liddell, J.E. 1996. Effects of cultivation techniques and methods of straw disposal on predation by *Pterostichus melanarius* (Coleoptera: Carabidae) upon slugs (Gastropoda: Pulmonata) in an arable field. *Journal of Applied Ecology*, 33, pp.741-753.

Symondson, W.O.C., Cesarini, S., Dodd, P.W., Harper, G.L., Bruford, M.W., Glen, D.M., Wiltshire, C.W. and Harwood, J.D. 2006. Biodiversity vs. biocontrol: positive and negative effects of alternative prey on control of slugs by carabid beetles. *Bulletin of Entomological Research*, 96, pp.637-645.

Tackenberg, M., Volkmar, C., Schirrmann, M., Giebel, A. and Dammer, K.H. 2018. Impact of sensor-controlled variable-rate fungicide application on yield, senescence and disease occurrence in winter wheat fields. *Pest Management Science*, 74, pp.1251-1258.

Tan, K. 2005. *Soil sampling, preparation, and analysis.* Second edition. Florida: CRC Press.

Taylor, L.R. 1961. Aggregation, variance and the mean. *Nature*, 189, p.732.

Taylor, L. R. 1984. Assessing and interpreting the spatial distributions of insect populations. *Annual Review of Entomology,* 29, pp.321- 357.

The Andersons Centre. 2014. *Crop production technology; the effect of the loss of plant protection products on UK agriculture and horticulture and the wider economy.* [ONLINE] NFU. Available at: https://www.nfuonline.com/andersons-final-report/ [Accessed 7th February 2016]

The Crop Residues (Burning) Regulations 1993. SI 1993/1366.

Thomas, D.C. 1947. Some observations on damage to potatoes by slugs. *Annals of Applied Biology*, 34, pp.246-251.

Thorbek, P. and Topping, C.J. 2005. The influence of landscape diversity and heterogeneity on spatial dynamics of agrobiont linyphiid spiders: an individual-based model. *Biocontrol*, 50, pp.1-33.

Triebskorn, R.A., Henderson, I.F. and Martin, A.P. 1999. Detection of iron in tissues from slugs (*Deroceras reticulatum* (Müller)) after ingestion of iron chelates, by means of energy-filtering transmission electron microscopy (EFTEM). *Pesticide Science*, 55, pp.55-61.

Twining S, Clarke J, Cook S, Ellis S, Gladders P, Ritchie F, Wynn S, ADAS. 2009. *Pesticide availability for potatoes following revision of Directive 91/414/EEC: Impact assessments and identification of research priorities. Project report 2009/2*. [ONLINE] AHDB. Available at: http://www.potato.org.uk/sites/default/files/publication_upload/20092_Pesticide_availability_Report_R415_0.pdf [Accessed 15th June 2019]

Uchmański, J. and Grimm, V. 1996. Individual-based modelling in ecology: what makes the difference? *Trends in Ecology & Evolution*, 11, pp.437-441.

UK Plant protection Products (Sustainable Use) Regulations 2012. SI 2012/1657.

Van den Akker, J.J.H. and Soane, B. 2005. *Compaction*. In: Rattan, L. *ed*, Encyclopaedia of Soil Science. 2nd Edition. Marcel Dekker, New York. Pp. 285-293.

Verbist, K., Torfs, S., Cornelis, W.M., Oyarzún, R., Soto, G. and Gabriels, D. 2010. Comparison of single-and double-ring infiltrometer methods on stony soils. *Vadose Zone Journal*, 9, pp.462-475.

Voluntary Initiative Steering Group. 2002. *First annual report of the Voluntary Initiative Steering Group.* [Online] Voluntary Initiative. Available at: http://www.voluntaryinitiative.org.uk/media/1027/first-annual-report.pdf [Accessed 6th September 2016]

Walters, K.F.A..and Cherrill, A. 2018. Response to declining availability of Plant Protection Products: A central role for vegetation management. *Aspects of Applied Biology,* 139, pp.103-114.

Want, R. 2006. An introduction to RFID technology. *IEEE Pervasive Computing*, 1, pp.25-33.

Wäreborn, I. 1970. Environmental factors influencing the distribution of land molluscs of an oligotrophic area in southern Sweden. *Oikos*, 21, pp.285-291.

Wareing, D. and Bailey, S. 1985. The effects of steady and cycling temperatures on the activity of the slug *Deroceras reticulatum. Journal of Molluscan Studies*, 51, pp.257-266.

Wareing, D. 1986. Directional trail following in *Deroceras reticulatum* (Müller). *Journal of Molluscan Studies,* 52, pp.256-258.

Willis, J. C., Bohan, D. A., Powers, S. J., Choi, Y. H. and Gussin, E. 2008. The importance of temperature and moisture to the egg-laying behaviour of a pest slug, *Deroceras reticulatum*. *Annals of Applied Biology*, 153, pp.105-115.

Wilson, M.J., Glen, D.M. and George, S.K. 1993. The rhabditid nematode *Phasmarhabditis hermaphrodita* as a potential biological control agent for slugs. *Biocontrol Science and Technology*, 3, pp.503-511.

Wilson, M.J., Glen, D.M., George, S.K. and Hughes, L.A. 1995. Biocontrol of slugs in protected lettuce using the rhabditid nematode *Phasmarhabditis hermaphrodita*. *Biocontrol Science and Technology*, 5, pp.233-242.

Wójtowicz, M., Wójtowicz, A. and Piekarczyk, J. 2016. Application of remote sensing methods in agriculture. *Communications in Biometry and Crop Science*, 11, pp.31-50.

Wood, C. 2006. *Countryside Survey 2007(Soils) Preparatory Phase II; Soil bulk density sampling.* [ONLINE] NERC. Available at: http://nora.nerc.ac.uk/id/eprint/503786/ [Accessed 10th March 2019]

Yildirim, M.Z. and Kebapçi, Ü. 2004. Slugs (Gastropoda: Pulmonata) of the Lakes Region (Göller Bölgesi) in Turkey. *Turkish Journal of Zoology*, 28, pp.155-160.

Young, A.G. 1990. Assessment of slugs using bran-baited traps. *Crop Protection,* 9, pp.355-358.

Young, A.G. and Port, G.R. 1991. The influence of soil moisture on the activity of *Deroceras reticulatum* (Müller). *Journal of Molluscan Studies*, 57, pp.138-140.

Young, A. G., Port, G. R., Emmett, B. J., and Green, D. B. 1991. Development of a forecast of slug activity: models to relate slug activity to meteorological conditions. *Crop Protection*, 10, pp. 413-415.

Young, A.G., Port, G.R. and Green, D.B. 1993. Development of a forecast of slug activity: validation of models to predict slug activity from meteorological conditions. *Crop Protection*, 12, pp.232-236.

Zamparas, M. and Zacharias, I. 2014. Restoration of eutrophic freshwater by managing internal nutrient loads. A review. *Science of the Total Environment*, 496, pp.551-562.